Docket No.: 1010/16959-US4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

HOWARD L. **WEINER** *et al.*

Serial No.: 08/469,492   Art Unit: 1645

Filed: June 6, 1995   Examiner: P. Duffy

For: **BYSTANDER SUPPRESSION OF AUTOIMMUNE DISEASES**

- -

DECLARATION OF MATTHIAS G. VON HERRATH

Hon. Commissioner of
  Patents and Trademarks
Washington, DC   20231

Sir:

Matthias G. Von Herrath declares and states as follows:

BACKGROUND INFORMATION

1.   I hold both a Medical Doctor and a Doctor of Philosophy degree, conferred by

Freiburg Medical School in 1988. Currently, I am employed as Associate Professor

by The Scripps Research Institute and I have held this position since 1999.

Previously, I was Assistant Professor at the same institution from 1996 to 1999.

For the past ten years, I have conducted extensive research in the immunology and

immunomodulation of autoimmune diseases and particularly insulin dependent

diabetes mellitus (IDDM).

2.   I am an author of over 30 publications on the immunology of autoimmune

diseases including several articles on bystander suppression. Further details of my

qualifications are provided in the attached copy of my curriculum vitae (Attachment

1).

3.   I submit this declaration in support of the patentability of the application

identified above. I am not a co-inventor of this application and I hold no financial

interest in either the Brigham & Women's Hospital or AutoImmune, Inc. which I

understand have certain rights in the invention.

4.   I am familiar with this application, including its specification, currently

pending claims (Attachment 2), Office Action of March 29, 2000 and references

cited therein, namely Tobin US patent 5,475,086; Herbert et al The Dictionary of

Immunology, Fourth Edition, Academic Press 1995, pp17 and 88; Mueller, D.L.

The Journal of NIH Research, 6:47-51, 1994; Hafler et al J. Immunol. 139:68,

1988; Cohen, I. et al AutoImmune Disease Models, p.2; and Paul et al Eds

"Fundamental Immunology" p.548.

5.   Briefly, the claimed invention involves administration of a bystander antigen to suppress an autoimmune response associated with the autoimmune disease, i.e., to induce tolerance. The claims further specify that the bystander antigen is an antigen to which T-cells that modulate an abnormal autoimmune response are NOT sensitized, and that the mode of its administration is by mouth or nasal route.

QUESTIONS CONSIDERED

6.   I understand that the United States Patent & Trademark Office has rejected the claims as non compliant with certain statutory requirements. Specifically, I understand that the Examiner is of the opinion that the patent application does not describe the invention as claimed in sufficient detail to enable a person of ordinary skill in the field of the invention to use it for all autoimmune diseases and bystander antigens encompassed by the claims.

7.   I have been retained by patent counsel to consider the following questions objectively from the point of view of a person of ordinary skill in the field at the time this application was first filed, which I am told was February 28, 1992. These questions are:

QUESTION I: Does the specification provide sufficient support for the claims by circumscribing the area of application of the claimed invention and providing a representative number of working embodiments? Or does the fact that many of the antigens disclosed in the specification (and employed in experiments described therein) can be autoantigens detract from the generality of the statements in the specification such that a person of ordinary skill in the field would consider the invention to have been demonstrated only for glucagon and insulitis and for no other pure bystander antigen or autoimmune disease?

QUESTION II: On February 28, 1992, would a person of ordinary skill in the field reasonably expect the claimed invention to be operative in humans afflicted with various autoimmune diseases based on the animal model data presented in the specification? Based on the teachings of the specification and the general knowledge in the field at the time (2/28/92), would such a person know which bystander antigen to administer, when to begin, how much to administer, how often and for how long, or would such a person have to conduct an inordinate amount of experimentation--beyond what is considered routine experimentation in this field--in order to obtain this information?

QUESTION III: Are the admonitions in Mueller et al, *supra*, at p.49-50 regarding anergy induction applicable to the method of the present claims, i.e., bystander suppression?

Question I

8.   I disagree with the Examiner on this question. The specification, through

the experiments it describes, makes clear to a person of ordinary skill in the field

that bystander suppression does not require administration of an antigen to which

activated T-cells of the host are sensitized, but results in suppression of the

Docket No. 1010/16959-US4

-4-

autoimmune (or autoimmune-like) response even when the administered antigen is a "pure bystander." A "pure bystander" is an antigen that is expressed in an organ or tissue that is the target of an abnormal autoimmune (or autoimmune-like) response but is not itself a target of the abnormal autoimmune response. The specification provides data with the following "pure bystander" antigens:

- glucagon orally administered to NOD mice (p48, lines 10-27 and p.49, Table 5)

- MBP orally administered to mice immunized with PLP (Example 6, p.49 lines 22-34, p.50, lines 1-13)[1]

- Ovalbumin orally administered to mice immunized with MBP (Example 2, pp36-45, especially p.42, line 6 through p.44 line 35)[2]

---

[1]Even though MBP can induce EAE, in this experiment, PLP only was used for EAE induction. Moreover, the mice used for the experiment had to be healthy and would not have activated T-cells that would recognize MBP. Hence, MBP in this experiment fits the definition of bystander antigen that appears in claim 37.

[2]Ovalbumin ("OVA")cannot induce EAE. It is thus clearly a bystander antigen within the definition of claim 37. Moreover, ovalbumin is not expressed in the organ or tissue afflicted in EAE (which is the central nervous system) and is thus totally removed from the locus of autoimmune response. It follows that if OVA can induce bystander suppression, such suppression is a general immune phenomenon

- Noninducing fragments of MBP orally administered to mice challenged with MBP (Example 3, pp 45-46 and p.29, lines 5-10).

- Peptides derived from insulin which are not recognized by T-cells of NOD mice (p.48, lines 24-26 and p.49, Table 5).

9.   The specification thus details experiments with at least five different "pure bystander" antigens (i.e. antigens not targeted by the immune response to be suppressed) administered in two different disease models. In particular, the use of OVA to suppress MBP-induced EAE is an important indication of the general applicability of the concept of bystander suppression because under normal circumstances (and unlike MBP and PLP) OVA is neither specific to the organ targeted by the aggressive autoimmune response, nor itself the target of an autoimmune or autoimmune-like response. In the OVA experiment detailed in the specification, Weiner et al engineered the OVA to be concentrated at the locus of the induced autoimmune-like response by injecting OVA into the mice at the site of their immunization with MBP, and thus created an artificial antigen "expressed" in

---

independent of the context of a particular autoimmune response or autoimmune disease or animal model of such disease, as I discuss below.

the "target organ" where the abnormal immune response occurred. The inventors did this to show that regulatory T cells (which were elicited in the mice by feeding them OVA) "home-in" to the site of inflammatory immune response (where the OVA, which the regulatory cells recognize was artificially introduced) and reduce this response. Unlike MBP or PLP, OVA is not associated with any autoimmune disease since immunization with OVA does not cause disease; unlike glucagon, OVA is not even specific to an organ targeted by an abnormal autoimmune response. Thus, even though Weiner et al did not test their method on *every* autoimmune disease, in my opinion, a person of ordinary skill in the field would consider applicability of their method in any autoimmune disease to be likely in view of the experiments they did conduct and especially the OVA experiment: if immune regulation results by feeding an animal an antigen unrelated to any abnormal autoimmunity, and by mimicking a tissue-specific antigen through injection of this antigen to a site where autoimmune (or autoimmune-like) response is taking place, it follows that the immunoregulatory phenomenon observed by Weiner et al using bystander antigens is very likely a general one.

10.   Even without taking into account these implications of the OVA experiment, the inventors have shown bystander suppression for four different antigens in two different autoimmune disease models (EAE and NOD diabetes), which in my view

Docket No. 1010/16959-US4

-7-

supports the proposition that bystander suppression would abate autoimmunity in autoimmune diseases in general. Most important, the inventors have shown the regulatory immune response following ingestion of the bystander antigen to be associated with expression by regulatory T cells of nonspecific immunoregulatory factors, such as TGF-β. These factors are associated with the regulatory nature of the response and not with the nature of the antigen which the regulatory T cells recognize. This finding lends additional support to the generality of bystander suppression as elucidated by Weiner et al.

11. Thus, as a result of the experimental evidence contained in the specification, in February 1992, a person of ordinary skill in the field would have expected "pure" bystander suppression by oral or nasal route to be an effective means of suppressing autoimmune response regardless of the identity of the bystander antigen and regardless of the particular experimental system used. The experiments in the specification indicated that bystander suppression is a general immune phenomenon, even apart from the context of autoimmunity (see for example the experiment with OVA). When these experiments were presented to the scientific community, they were at first surprising but they were quickly recognized and have since become generally accepted as having elucidated an important and general immunoregulatory mechanism.

12.   The experiments in the specification would have indicated to a person of ordinary skill in the field that bystander suppression using a "pure" bystander antigen is T-cell mediated suppression and, more likely than not, is effected in a manner similar to oral (and more generally mucosal) tolerance using autoantigens. In both forms of tolerance induction, ingestion of the antigen is accompanied by a regulatory rather than an aggressive immune response following antigenic stimulation. Hence, the person of ordinary skill would have expected bystander suppression to be effective when the bystander antigen is administered through the nasal mucosa, as the specification indicates at p.24, lines 25-29, similar to when an autoantigen is fed or nasally administered.

13.   Thus, in my opinion, in 1992, the person of ordinary skill would have believed the statements in the specification and claims that "pure" bystander antigens by oral or nasal route would be effective to suppress an autoimmune response in humans.

Question II

14.   Given the data in the specification, it is my opinion that a person of ordinary skill would clearly expect that oral or nasal administration of a "pure" bystander antigen (i.e., an antigen not recognized by activated auto-aggressive T cells of the patient) would suppress an abnormal autoimmune response in humans. The skilled

person would know from the specification that such bystander antigens can be

selected from among peptide fragments of autoantigens which are not recognized

by activated human T-cell clones. In my opinion, the assay for this purpose was

feasible in 1992. (See, for example Hafler et al, J. Immunol. 139:68-72, 1987.)

The amount of experimentation required would not be considered excessive

because, in immunology, this type of experimentation was and is routine.

15.   The specification provides ample guidance as to how much bystander antigen

to use as a starting point. For example, at p.25, lines 3-6, the specification

provides a general range of 0.1mg-15mg/kg daily for nasal administration; at pp.26-

27, the specification allows for developmental work which in 1992 could have

been used to refine the dosages to be given to humans. The specification also

provides detailed guidance in the form of dosages for PLP-induced disease at p.17,

and even allows for differences between species of hosts. The fact that the

various antigens may be autoantigens in certain individuals or experimental contexts

would not alter the value of this disclosure. In other words, the effective dosage

range of a bystander antigen which can also be an autoantigen can be used as

guidance for the effective dosage range of a "pure" bystander. The principle behind

use of each is the same: an antigen is administered mucosally in order to regulate

immune response. The immunoregulatory effect in both instances is due to

Docket No. 1010/16959-US4

elicitation of regulatory cells, and expression of regulatory factors, such as TGF-β.

All of this was known with respect to autoantigens administered orally or nasally to achieve tolerance, as evidenced for example from the attached references: *Zhang, Z. J., et al*, ("Suppression of diabetes in Nonobese Diabetic Mice by Oral Administration of Porcine Insulin," *P.N.A.S. (USA), 88*:10252-10256, November 1991 at p.10255); *Miller, A. et al* ("Tolerance and Suppressor Mechanisms in Experimental Autoimmune Encephalomyelitis: Implications for Immunotherapy of Human Autoimmune Diseases," *FASEB, 5*:2560-2566, Aug.1991, at p. 2562, right col.); and *Miller A., et al*,"Suppressor T Cells generated by Oral Tolerization to Myelin Basic protein Suppress both In Vitro and In Vivo Immune Responses by the Release of Transforming Growth Factor β after Antigen-Specific Triggering," *P.N.A.S. (USA), 89*:421-425, January 1992, at pp. 424-425).[3] In light of the experiments described in the Weiner et al specification, it became likely that these conclusions could be extended to bystander antigens. Copies of the references cited in this Paragraph 15 are attached as Attachment 3.

QUESTION III

---

[3]It was also known in 1992 that an antigen could be administered orally or nasally indefinitely as long as benefits persisted, it being well-tolerated by treated subjects.

16.   I find the disclosure and admonitions of Mueller inapplicable to the claimed

invention. Mueller is concerned exclusively with the concept of anergy, which is a

totally different immunological concept from bystander suppression. Anergy can

only be induced when an activated T-cell encounters (under certain circumstances,

namely in the absence of co-stimulatory factors) the very antigen against which it is

directed. I am not aware of any other antigen that can induce anergy for such a T-

cell. Accordingly, if it is desired to suppress an immune response by anergizing the

T-cells that mediate it, each such T-cell must be exposed to the very antigen that it

recognizes. It is therefore necessary to know the specificity of each T-cell in order

to administer the proper antigen. It is also necessary to ensure the absence of co-

stimulation. If co-stimulation is present, an aggressive immune response can ensue

instead of immune regulation; hence the extreme caution permeating the

statements in Mueller.

17.   Anergy is thus totally different from bystander suppression which can

proceed by administration of an antigen that does not constitute a T-cell target of

the aggressive response to be suppressed, and without fear of co-stimulation.

Moreover, bystander suppression takes place because the elicited regulatory T-cells

which recognize the administered antigen secrete nonspecific immunosuppressive

factors, such as TGF-β , which suppress immune responses at the locus of their

secretion without regard to the specificity of the activated T-cells that may be found in such locus. For these reasons, the statements in Mueller, while applicable to anergy, are inapplicable to bystander suppression.

18.   I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: _October 24th 2000_

Matthias G. Von Herrath

Docket No. 1010/16959-US4

Tolerance and suppressor mechanisms in experimental autoimmune encephalomyelitis: implications for immunotherapy of human autoimmune diseases

ARIEL MILLER, DAVID A. HAFLER, AND HOWARD L. WEINER

Center for Neurologic Diseases, Division of Neurology, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA

ABSTRACT Recent advances in understanding antigen recognition at the level of the trimolecular complex have provided new approaches for selective immunotherapy. Many of these approaches have been applied successfully to the animal model experimental autoimmune encephalomyelitis, and some are being tested in the human disease multiple sclerosis. In addition, new approaches utilizing nonspecific modulation of immune function are being explored in animals and humans. Immunospecific therapy in autoimmune diseases will ultimately be based on understanding how the normal immune system maintains unresponsiveness to self and how this state of self-tolerance is broken. Strategies for specific immune intervention in human diseases based on components of the trimolecular complex will have to take into account the polymorphism of the major histocompatibility complex in humans and the degree of heterogeneity among autoimmune T cells that react with an autoantigen.— Miller, A.; Hafler, D. A.; Weiner, H. L. Tolerance and suppressor mechanisms in experimental autoimmune encephalomyelitis: implications for immunotherapy of human autoimmune diseases. *FASEB J.* 5: 2560-2566; 1991.

Key Words: autoimmunity · immunotherapy · experimental autoimmune encephalomyelitis · tolerance

THE IMMUNE SYSTEM IS CONFRONTED BY a variety of molecules, among which it must discriminate between self and non-self. Immunological tolerance is the acquisition of unresponsiveness to self-antigens, and as such is essential for preservation of the integrity of the host. Understanding the mechanisms of tolerance has become a central issue in immunology, especially in relation to ways in which breakdown of self-tolerance that results in autoimmune diseases can be prevented or reversed.

Self-tolerance is not preprogrammed in the germline but is acquired somatically by mechanisms that delete or inactivate autoreactive clones. Mechanisms of self-tolerance to T cells include clonal deletion, clonal anergy, and active suppression (Table 1). Since the first description of acquired immunological tolerance (1), a variety of experimental systems have been used to define the extent to which each of these mechanisms contributes to developing and maintaining self-tolerance. There is direct evidence that clonal deletion of autoreactive T cells within the thymus is one of the primary mechanisms responsible for maintaining immunological self-tolerance (2, 3). Clonal deletion occurs at the CD4⁺CD8⁺ double-positive or single-positive stage of T cell development in the thymus, and involves participation of the CD8 or CD4 molecules in recognition of major histocompatibility com-

plex (MHC)[1] class I or class II molecules. Nonetheless, clonal deletion does not remove all autoreactive T cells, especially for tissue-specific antigens (e.g., components of the eye, brain, or testes), which are not expressed in the thymus. Immunological unresponsiveness to sequestered tissue antigens such as brain antigens may be attributed partially to the location of these self-antigens behind natural anatomical barriers or to the lack of expression of class I or class II MHC molecules in the target organ. Furthermore, autoreactive T cells may be down-regulated or suppressed either in the periphery or in the thymus by encountering antigen in the absence of MHC or another costimulatory signal (or signals) from antigen-presenting cells (APCs) leading to functional inactivation but not deletion of autoreactive T cells (clonal anergy) (4–6). Finally, autoreactive cells may be actively suppressed by regulatory or suppressor T cells that inactivate or lyse autoreactive cells in a manner that is not yet precisely defined (7–11).

EAE AS A MODEL FOR ORGAN-SPECIFIC AUTOIMMUNE DISEASE

Elucidation of mechanisms underlying human diseases and design of immunotherapy is often facilitated through the study of animal models. Because of similarities in both clinical expression and pathology, experimental autoimmune encephalomyelitis (EAE) has been used as the primary model for multiple sclerosis (MS), a human demyelinating disease of the central nervous system (CNS) of presumed autoimmune etiology (12). Experimental autoimmune encephalomyelitis is an inflammatory disease of the CNS manifest in rodents either as an acute or chronic relapsing process. Immunization of animals with myelin basic protein (MBP) plus complete Freund's adjuvant stimulates a population of autoreactive CD4⁺ T cells that migrate to the CNS and cause disease. Myelin-specific CD4⁺ T cell lines and clones can be generated, and upon adoptive transfer will cause disease in naive recipients (13). Myelin basic protein has been the CNS encephalitogen most extensively studied, although more recently it has become clear that proteolipid protein (PLP) is also a major CNS encephalitogen (14). The encephalitogenic epitopes of MBP differ among animal strains and are re-

---

[1]Abbreviations: MHC, major histocompatibility complex; APCs, antigen-presenting cells; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; CNS, central nervous system; MBP, myelin basic protein; PLP, proteolipid protein; MSCH, mouse spinal cord homogenate; TCR, T cell antigen receptor; CFA, complete Freund's adjuvant.

TABLE 1. *Mechanisms of immunologic tolerance*

	References
Clonal deletion	
Negative selection by MHC molecules during thymic development	2, 3
Clonal anergy or functional inactivation	
Lack of costimulatory signal by cell which presents antigen resulting in an inability to make IL 2	4-6
Active suppression	
Regulatory T cells with cytotoxic activity or that suppress via soluble factors or noncytolytic cell contact	7-11

stricted by MHC class II molecules, predominantly I-A, and in a few instances I-E (15, 16).

FACTORS ASSOCIATED WITH AUTOIMMUNITY AND SUSCEPTIBILITY TO EAE

Certain animal strains are resistant to development of EAE, and susceptibility is linked to MHC genes (17, 18). A similar situation occurs in humans in which certain HLA types are associated with specific autoimmune diseases, e.g., multiple sclerosis (DR2, DQW1), diabetes (DR3, DR4), and rheumatoid arthritis (DR4) (19). The association between autoimmune diseases and particular MHC alleles suggests that T cells restricted by MHC gene products are involved in the disease process. Further support for the role of T cells in autoimmune diseases is the association with certain T cell receptor β-chain alleles (20).

The mechanism by which MHC molecules are associated with susceptibility to autoimmune diseases is unclear, but probably relates to preferential presentation of autoantigens to T cells and perhaps preferential stimulation of certain classes of T cells. However, resistance or susceptibility to EAE and other autoimmune processes is clearly a multigenic process, with genes outside the MHC complex influencing susceptibility (21).

It has been shown that astrocytes can present myelin basic protein to encephalitogenic T cell lines in vitro (22), and that bone marrow-derived perivascular microglial cells-in-the CNS-present antigen in vivo (23). In vivo studies demonstrated the requirement for histocompatibility between invading encephalitogenic T cells and perivascular microglial cells for induction of EAE (23). Recent observations suggest that among immunological activities of the cells of the CNS, resistance or susceptibility to autoimmune processes may be related in part to the ability of brain endothelia or glia cells from susceptible strains of rats or mice to express sufficient levels of MHC to stimulate autoreactive T cells (24). These differences in stimulating capacity between species appear tissue-specific as non-glial cells from various strains had equivalent abilities to stimulate T cell lines. These observations raise the possibility that MHC antigens on glial and non-glial tissue are different, and such a difference could be important in determining T cell-mediated, organ-specific autoimmunity. Aberrant MHC expression has also been postulated to play a role in endocrine autoimmunity (25). Furthermore, in addition to presentation of antigen to the cell receptor by antigen-presenting cells, costimulatory signals are required for T cell triggering, and T cell anergy can develop if antigen is presented to T cells in the absence of such signals (4-6). It is possible that antigen-presenting cells in the CNS constitutively anergize autoreactive T cells that migrate to the CNS by not being able to provide the necessary costimulatory signals.

EAE is induced by injection of MBP in adjuvant. The inducing events in a spontaneously occurring autoimmune disease such as multiple sclerosis are unknown. Presumably, the immune system is triggered by environmental factors such as viruses and bacteria. Possible mechanisms include infection of CNS tissue by a virus with release of autoantigen (26), molecular mimicry in which portions of a virus are homologous to CNS proteins (27, 28), or general activation of the immune system through cytokines such as γ-interferon, which are released in association with viral infection (26) (Table 2). Multiple sclerosis patients with an increased number of clinically evident viral infections have more attacks (29), and treatment of MS with γ-interferon made the disease worse (30).

CHRONIC RELAPSING EAE (CR-EAE)

EAE may be an acute, self-limiting process or a chronic, relapsing disease. The development of chronic as opposed to acute EAE relates to a number of factors, including the animal strain injected and the nature of the inoculation. In the Lewis rat, subcutaneous or intradermal injection of MBP plus adjuvant leads to an acute paralytic disease 10-17 days after injection, with recovery. Recovered animals are resistant to subsequent induction of EAE. Postrecovery suppressor cells of the CD4$^+$ phenotype that inhibit antigen-specific production of IFN-α by encephalitogenic T cells appear related to the recovery of Lewis rats from acute EAE and to the inability to reinduce EAE by active immunization in recovered animals (32). However, these Ag-specific suppressor cells do not inhibit disease after passive transfer of activated MBP-specific T cells, which suggests that they act at the afferent rather than the efferent phase of the disease. The existence of postrecovery suppressor cells raises the theoretical question of whether in humans natural resistance to autoimmune processes is acquired. It is possible that humans

TABLE 2. *Factors associated with autoimmune diseases*

	References
Genetic susceptibility	
Association of autoimmune diseases with MHC alleles	19
Non-MHC genes involved in disease susceptibility	20, 21
Triggering events	
Infection or damage to target organ with release or alteration of autoantigens	26
Molecular mimicry or cross-reactivity between virus or bacteria and self protein	27, 28
Abberant expression of MHC moleculesl by local antigen-presenting cells in target organ with stimulation of autoreactive T cells	24-25
Non-antigen-specific activation of the immune system by cytokines released after systemic infection	29, 30
Drugs	31
Dysregulation of the immune system	
Defective generation of suppression	36
Altered neuroendocrine regulation	33-35

oecome naturally protected against autoimmune processes by having self-limited or transient subclinical autoimmune attacks, and autoimmune disease develops when there is a defect in developing such resistance. Spontaneous recovery of Lewis rats from EAE depends on regulation of the immune system by endogenous adrenal corticoids (33). A defective hypophysis-pituitary-adrenal axis response to inflammation results in low levels of endogenous corticosteroids and may contribute to susceptibility of the Lewis rat to EAE and other autoimmune diseases such as streptococcal cell wall arthritis (34).

In multiple sclerosis and other autoimmune diseases such as rheumatoid arthritis, defects in antigen nonspecific immunoregulation have been identified, and such defects could contribute to generation of a relapsing/chronic immune response (36). A mild relapsing form of EAE can be triggered in Lewis rats when they are injected with whole spinal cord homogenate (37). CR-EAE is easily produced in the mouse either by injection of white matter homogenate in complete Freund's adjuvant (CFA) or the transfer of MBP reactive T cell clones (38). Immunoregulatory mechanisms associated with CR-EAE are poorly understood. One important principle related to relapsing disease is that the myelin antigen or the determinant on the myelin antigen associated with the chronic disease may be different from the determinant or antigen that induces the initial attack (39). For example, relapsing disease induced by MBP or an MBP clone might depend on induction of a cellular or humoral immune response to another myelin antigen, such as proteolipid protein (40), myelin oligoclondrocyte glycoprotein (41), or other myelin antigens. If this is true, it has important implications for designing therapy related to antigen-specific modulation of any component of the trimolecular complex.

IMMUNOTHERAPY BASED ON SPECIFIC MODULATION OF THE TRIMOLECULAR COMPLEX

Antigen recognition by T cells involves interaction of three components: antigen, the T cell receptor, and MHC. To down-regulate antigen-specific immune responses, each component is a potential target for immunotherapy (Fig. 1).
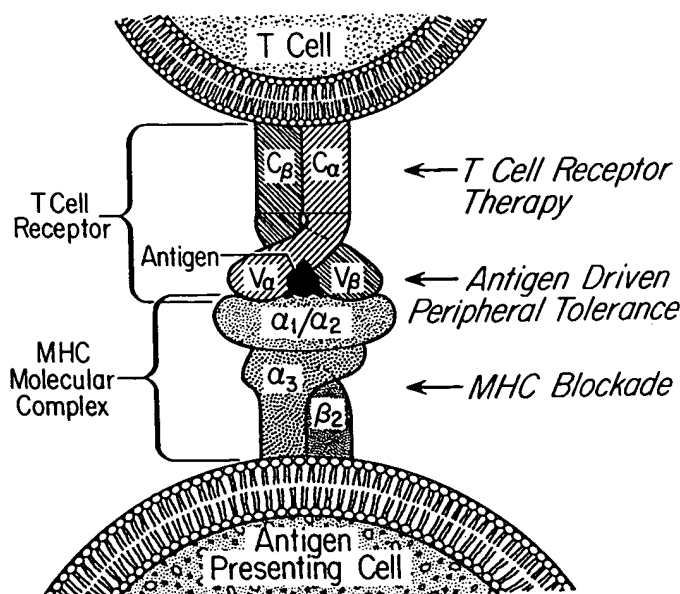

Figure 1. The trimolecular complex as a target for immunotherapy.

Indeed, successful antigen-specific immunotherapy in EAE has been achieved by using or affecting the function of each component of the trimolecular complex (Table 3). Some of these approaches are being tested in clinical trials in MS and other autoimmune diseases.

ANTIGEN-DRIVEN PERIPHERAL TOLERANCE

When the immune system is confronted by a foreign antigen, two pathways are possible: tolerance or immunity. Whether tolerance or immunity develops depends on many factors, including the route of administration, whether adjuvants are used, and the concentration and physical state of the antigen. A variety of methods have been used to suppress EAE using antigen-driven tolerance, including administration of antigen intravenously, in incomplete Freund's adjuvant, in liposomes coupled to autologous splenocytes, and orally (42–49). We have been investigating oral tolerance as a method to down-regulate both experimental and human autoimmune disease because of its inherent clinical applicability.

Oral administration of antigens was the first demonstration of immune tolerance and was reported in 1911 by Wells, who found that guinea pigs fed hen's egg protein lose the ability to develop anaphylactic sensitivity to the ingested protein (50). In 1946, Chase reported suppression of responses to haptens after oral administration (51). Oral immunization induces local B cell responses in the gut mucosa and at the same time tolerizes for systemic administration of the same antigen. Although the exact mechanism of suppression associated with oral tolerance is not defined, many experiments demonstrate that active suppression mediated by T cells occurs after oral administration of antigen (52). Antigen feeding affects both humoral and cellular immune responses but has more profound effects on cellular immunity.

Some investigators have suggested that after antigen is orally administered, CD4+ suppressor/inducer cells are generated in Peyer's patches, and these cells then migrate to mesenteric lymph nodes and the spleen where they down-regulate systemic immune responsiveness by inducing CD8+ suppressor-effector cells (53). Furthermore, it has been reported that intestinal epithelial cells may preferentially activate CD8+ suppressor T cells (54).

Although oral tolerance has been studied for many antigens, it has only recently been applied to the study of autoimmune diseases. We and others have demonstrated that orally administered myelin basic protein and myelin antigens suppress acute and chronic-relapsing EAE (9, 44–46), S-Ag suppresses experimental autoimmune uveitis (55), and collagen II suppresses both collagen and adjuvant-induced arthritis (56–58). Suppression of EAE is related to the generation of antigen-specific CD8+ T cells that can adoptively transfer protection against EAE (9) and act via the release of the suppressive cytokine TGF-β after being triggered by antigen (59). We have also found that orally administered nonencephalitogenic fragments of MBP can suppress EAE. Thus, antigen-driven peripheral tolerance may involve suppressor epitopes that are different from immunodominant epitopes on the antigens that trigger CD4+ cells. This phenomenon has previously been shown in the HEL system (60) and in the EAE model (61). It is not clear why certain epitopes of a protein may have suppressive properties. Nonetheless, these findings raise the possibility that nonencephalitogenic fragments of an autoantigen such as MBP could be used to treat a disease such as MS, although different suppressor epitopes may be active in different individuals. A clinical trial

TABLE 3. *Immunotherapy based on specific modulation of the trimolecular complex*

	References
Antigen-driven tolerance	
Oral tolerance	9, 44–46, 55–58
Antigen coupled to autologous cells	47–49
Anti-TCR immunotherapy	
Anti-TCR monoclonal antibodies	67, 68
T cell vaccination	65, 66, 75
T cell receptor peptides	70, 71
MHC blockade	
MHC-blocking peptides	76–78
Anti-MHC class-II antibodies	79, 80

is currently in progress at our institution in which a myelin preparation is being orally administered to MS patients.

Another method to induce antigen-driven peripheral tolerance both in vitro and in vivo is by administration of antigen coupled to syngeneic lymphoid cells. In the EAE model, injection of mouse MBP-coupled splenocytes 7 days before immunization with mouse spinal cord homogenate (MSCH) prevents induction of acute EAE in SJL/J mice (47), and this approach also suppresses MSCH-induced CR-EAE (48). Neuroantigen-specific tolerance has been used to determine the relative role of MBP and PLP in MSCH-induced CR-EAE (49). It has been found that tolerization with PLP, not MBP-coupled spleen cells, prevented disease in these animals. These experiments demonstrate that antigen-driven tolerance can be used to determine which autoantigens are important in an autoimmune disease in which multiple antigens are potentially involved. In this regard we recently demonstrated that oral administration of type II collagen suppresses adjuvant arthritis (58), which suggests an important role for autoimmunity to CII in this model. In a similar fashion, a clinical trial in which antigen-driven tolerance is undertaken will in itself be a clinical experiment that will help define the degree to which immunity to particular autoantigen plays a pathogenic role in the disease.

A synthetic copolymer (Cop 1) that competes with MBP for binding to MBP reactive clones (62) suppresses EAE (63), and in a pilot trial subcutaneous administration demonstrated positive effects in patients with early, relapsing MS (64) although no effect was observed in the chronic form. The exact mechanism of action of Cop 1 in vivo is unclear. It was initially designed to generate antigen-driven tolerance due to its cross-reactivity to myelin basic protein, and is reported to induce MBP-specific suppressor cells (63). It is postulated to also act by specific inhibition of BP-specific effector cells.

ANTI-T CELL RECEPTOR THERAPY

EAE is mediated by CD4^{+} T cells specific for MBP or PLP. Cohen and co-workers (65) were able to suppress EAE by injecting MBP-specific T cell lines that were attenuated by irradiation, pressure, or glutaraldehyde treatment before immunizing animals with MBP plus CFA. The effect was for the most part antigen-specific in that injection of lines specific for another antigen had a minimal effect. This treatment has been termed T cell vaccination, and the presumed mechanism of action is an anticlonotypic or antiidiotypic response against the T cell antigen receptor (TCR) or other structures on the injected line or clone (66). Subsequently, it has been found that there is conservation of TCR among en-

cephalitogenic T cells, even in different animal models of EAE, despite known differences in MBP-epitope specificity and differences in major histocompatibility complex (MHC) restriction (67–69). These findings provided a more defined target for immunotherapeutic intervention. Two groups have reported successful treatment of EAE in the Lewis rat by immunizing with peptides comprising specific variable regions of the Vb8.2 T cell receptor (70, 71). Another successful approach has been the use of monoclonal antibodies directed against TCR structures used by encephalotogenic clones (67, 68). Nonetheless, anti-idiotypic antibodies directed against the T cell receptor of an MBP-specific T cell hybridoma have been reported to either suppress or enhance EAE (69). For anti-T cell receptor therapy to be successful in human disease, there must be limited heterogeneity of TCR usage by pathogenic autoreactive T cells. We have recently demonstrated restricted TCR usage for MBP clones from MS patients that recognize the immunodominant region of MBP, raising the possibility of T cell receptor therapy in MS (72). Furthermore, oligoclonal T cells have been described both in MS (73) and rheumatoid arthritis (74). Pilot trials of T cell vaccination using T cells clones from spinal fluid or joints in human autoimmune disease such as MS and rheumatoid arthritis are currently in progress (75). Other investigators are testing T cell receptor peptides in MS. Nonetheless, it is likely that with further study TCR usage in the recognition of autoantigens in autoimmune models and human diseases may not be strictly limited.

MHC-BLOCKING PEPTIDES AND ANTI-MHC MONOCLONAL ANTIBODY THERAPY

A third approach for immune intervention at the level of the trimolecular complex relates to the fact that autoantigens must be presented by MHC molecules and that there is an association between autoimmune diseases and particular MHC alleles (19). Based on recognition of the MBP peptide (P1-11), acetylated at its NH_2 terminal, as the immunodominant epitope that stimualtes encephalitogenic T cells in $H-2^u$ strain mice, it was possible to design peptides that competed for recognition of MBP at the level of antigen presentation without interacting with TCR to block T cell activation (76). By substitution of a single amino acid (lysine to alanine at position 4 of Acl-11), it was possible to generate peptides with increased affinity for the $I-A^u$ binding determinant. The same peptides substituted with other residues (glutamine to alanine in position 3) are not recognized and do not activate encephalitogenic T cells. Immunization of mice with both the analog peptide and the encephalitogenic peptide suppressed EAE compared with animals given the encephalitogenic peptide alone (77). Peptide blocking of MHC has also been shown for PLP-induced EAE by peptide 139-151 (78). To be effective, the MHC blocking peptide must compete with endogenous peptides for the MHC binding site and therefore must be continually supplied to avoid induction of the autoimmune response, which may not be feasible clinically.

EAE as well as other autoimmune diseases have been treated effectively by administration of anti-Ia monoclonal antibodies (79, 80). The mechanism of action of anti-Ia immunotherapy is unclear. Such therapy was designed to inhibit MHC presentation of Ag to T cells and offer the advantage of allelic-specific therapy, suppressing autoimmune responses linked to certain alleles without inducing global immune suppression. Investigators have also reported generation of T cells that adoptively transfer suppression after anti-Ia therapy (80, 81).

IMMUNOTHERAPY BASED ON MODULATION OF NONSPECIFIC IMMUNE FUNCTION (TABLE 4)

Monoclonal antibodies against T cell surface markers

Monoclonal antibodies that recognize T cell surface determinants not related to the TCR have been used in animals as immunosuppressive agents to reverse experimental models of autoimmune disease (82, 83). CD4⁺ T cells are the primary disease-inducing component in EAE. Both anti-pan T cell antibodies and anti-CD4 antibodies suppressed acute, and in some instances chronic, EAE (84). Another approach has been to target activated T cells in EAE (85), and anti-IL 2 receptor antibody has been shown to be effective in the NOD mouse model of diabetes (86). Interleukin 2 linked to diptheria toxin is another method to target activated cells (89). Pilot trials with murine monoclonal antibodies directed against T cell-surface antigens (CD4, CD2, and anti-T-12) have been reported in MS patients (87, 88). Immunologic effects were noted, but human anti-mouse responses prevented continued administration of these monoclonal antibodies for a chronic disease such as multiple sclerosis (88). Chimeric antibodies are expected to have less antigenicity, and thus may partially obviate the limitation of mouse mAb due to the human response against the mouse mAb constant regions. There are preliminary reports of positive effects of anti-CD4 antibodies in rheumatoid arthritis, and trials of chimeric anti-CD4 antibodies in MS patients are currently in progress. Another possible approach includes the use of monoclonal antibodies directed against adhesion molecules.

Other forms of therapy

Cytokines play an important role in the generation of immune responses, and a variety of approaches related to moderation of cytokine function or use of cytokines to alter immune reactivity are possible. As mentioned previously, MS was exacerbated by treatment with γ-interferon, presumably related to up-regulation of class II (30), and MS patients with increased numbers of viral infections have increased disease activity (29). Currently, there are multicenter trials utilizing β-interferon in MS based on the antagonistic effects of β-interferon on γ-interferon-induced Ia expression (90). Soluble IL 1 receptors are also being used to suppress immune responses (89).

Migration of activated lymphocytes into the target organ is the prerequisite for initiation of cell-mediated autoimmune responses. We have found rapid trafficking of in vivo-labeled T cells from peripheral blood to the cerebrospinal fluid in MS (91). It has been shown that traffic of T cells to target tissue is related to activation of the heparanase that degrades the heparan sulfate component of the subendothelial extracellular matrix. Expression of this heparanase and heparanase-dependent T cell traffic was shown to be inhibited in vitro and in vivo by heparinoid molecules, and suppression of experimental autoimmune diseases was demonstrated in animals by treatment with low doses of heparin (92). We performed a pilot trial of low-dose heparin (100–500 units/day) in chronic progressive MS but observed no positive effects (93).

Finally, chemical immunosuppression with drugs such as cyclophosphamide have been shown to be of benefit in some patients with MS (94) and in lupus nephritis. Methotrexate is efficacious in rheumatoid arthritis. Because of the multiple mechanisms involved in autoimmune responses, intermittent use of such drugs may be required even in the context of more specific modulation of the immune system discussed previously. ▣

REFERENCES

1. Billingham, R. E., Brent, L., and Medawar, P. B. (1953) Actively acquired tolerance of foreign cells. *Nature (London)* 172, 603–606
2. Kappler, J. W., Roehm, N., and Marrack, P. (1987) T-cell tolerance by clonal elimination in the thymus. *Cell* 49, 273–280
3. Kisielow, P., Bluthman, H., Staerz, U. D., Steinmetz, M., and von Boehmer, H. (1988) Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes. *Nature (London)* 333, 742–746
4. Ramsdell, F., Lantz, T., and Fowlkes, B. J. (1989) A nondeletional mechanism of thymic self-tolerance. *Science* 246, 1038–1041
5. Rammensee, H. G., Kroschewski, R., and Frangoulis, B. (1989) Clonal anergy induced in mature Vβ6⁺ T lymphocytes on immunized *MLs-1*ᵇ mice with *MLs-1*ᵃ expressing cells. *Nature (London)* 339, 541–544
6. Schwartz, R. H. (1990) A cell culture model for T lymphocyte clonal energy. *Science* 248, 1349–1356
7. Gershon, R. K., and Kondo, K. (1971) Infectious immunological tolerance. *Immunology* 21, 903–914
8. Sun, D., Qin, Y., Chluba, J., Epplen, J. T., and Wekerle, H. (1988) Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. *Nature (London)* 332, 843–845
9. Lider, O., Santos, L. M. B., Lee, C. S. Y., Higgins, P. J., and Weiner, H. L. (1989) Suppression of experimental allergic encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and in vitro immune responses is mediated by antigen-specific CD8⁺ T lymphocytes. *J. Immunol.* 142, 748–752
10. Salgame, P., Modlin, R. L., and Bloom, B. R. (1989) On the mechanism of human T cell suppression. *Int. Immunol.* 1, 121–129
11. Tada, T., Asano, Y., and Sano, K. (1989) Present understanding of suppressor T cells. *Res. Immunol.* 140, 291–294
12. Alvord, E. C., Kies, M. W., and Suckling, A. J., eds (1984) Experimental allergic encephalomyelitis a useful model for multiple sclerosis. In *Proceedings of Clinical Biology Research*, Vol. 146, Alan R. Liss Inc., New York
13. Ben-Nun, A., Wekerle, H., and Cohen, I. R. (1981) The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur. J. Immunol.* 11, 195–199
14. Tuohy, V. K., Lu, Z., Sobel, R. A., Laursen, R. A., and Lees,

TABLE 4. *Immunotherapy based on modulation of nonspecific immune function*

	References
Monoclonal antibodies directed against T cells	
Anti-CD4	82–84, 88
Anti-CD2	88
Down-regulation of activated cells	
Anti-IL 2 receptor monoclonal antibodies	85, 86
IL 2 toxin	89
Cytokine therapy	
β-Interferon	90
Soluble IL 1 receptors	89
Alteration of lymphocyte migration	91–93
Chemical immunosuppression	94

M. B. (1989) Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J. Immunol.* **142**, 1523-1527

15. Zamvil, S., Mitchell, D. J., Moore, A. C., Schwarz, A. J., Steifel, W., Nelson, P. A., Rothbard, J. B., and Steinman, L. (1987) T cell specificity for class II (I-A) and the encephalitogenic N-terminal epitope of the autoantigen myelin basic protein. *J. Immunol.* **139**, 1075-1079

16. Offner, H., Hashim, G. A., Celnik, B., Galang, A., Li, X., Burns, F. R., Shen, N., Heber-Katz, E., and Vandenbark, A. (1989) T cell determinants of myelin basic protein include a unique encephalitogenic I-E-restricted epitope for Lewis rats. *J. Exp. Med.* **170**, 355-367

17. Gasser, D. L., Newlin, C. M., Palm, J., and Gonatas, N. K. (1973) Genetic control of susceptibility to experimental allergic-encephalomyelitis in rats. *Science* **181**, 872-873

18. Lando, Z., Teitelbaum, D., and Arnon, R. (1980) Induction of experimental allergic encephalomyelitis in genetically resistant strains of mice. *Nature (London)* **287**, 551-552

19. Todd, J. A., Acha-Orbea, H., Bell, J. I., Chao, N., Fronek, Z., Jackob, C. O., McDermott, M., Sinha, A. A., Timmerman, L., Steinman, L., and McDevitt, H. O. (1988) A molecular basis for MHC class II-associated autoimmunity. *Science* **240**, 1003-1006

20. Seboun, E., Robinson, M. A., Doolittle, T. H., Cuilla, T. A., Kindt, T. J., and Hauser, S. L. (1989) A susceptibility locus for multiple sclerosis is linked to the T cell receptor β chain complex. *Cell* **57**, 1095-1100

21. Happ, M. P., Wettstein, P., Dietzschold, B., and Heber-Katz, E. (1988) Genetic control of the development of experimental allergic encephalomyelitis in rats. Separation of MHC and non-MHC gene effects. *J. Immunol.* **141**, 1489-1494

22. Fontana, A., Fierz, W., and Wekerle, H. (1984) Astrocytes present myelin basic protein to encephalitogenic T cell lines. *Nature (London)* **307**, 273-276

23. Hickey, W. F., and Hiromitsu, K. (1988) Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* **239**, 290-292

24. Massa, P. T., ter Meulen, V., and Fontana, A. (1987) Hyperinducibility of Ia antigen on astrocytes correlates with strain-specific susceptibility to experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* **84**, 4219-4223

25. Bottazzo, G. F., Pujol-Borrell, R., Hanafusa, T., and Feldmann, M. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* **2**, 1115-1118

26. Johnson, R. T. (1983) Persistent viral infections and demyelinated disease. In *Viruses and Demyelinating Diseases* (Mims, C. A., Cuzner, M. L., and Kelly, R. E., eds) pp. 7-19, Academic, New York

27. Fujinami, R. S., and Oldstone, M. B. A. (1985) Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* **230**, 1043-1045

28. Shaw, S. Y., Laursen, R. A., and Lees, M. B. (1986) Analogous amino acid sequences in myelin proteolipid and viral proteins. *FEBS Lett.* **207**, 266-270

29. Sibley, W. A., Bamford, C. R., and Clark, K. (1985) Clinical viral infections and multiple sclerosis. *Lancet* **1**, 1313-1315

30. Panitch, H. S., Hirsch, R. L., Schindler, J., and Johnson, K. P. (1987) Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. *Neurology* **37**, 1097-1102

31. Garraty, G., and Petz, L. D. (1975) Drug-induced hemolytic anemia. *Am. J. Med.* **58**, 398

32. Karpus, W. J., and Swanborg, R. H. (1989) CD4+ suppressor cells differentially affect the production of IFN-g by effector cells of experimental autoimmune encephalomyelitis. *J. Immunol.* **143**, 3492-3497

33. MacPhee, I. A. M., Antoni, F. A., and Mason, D. W. (1989) Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J. Exp. Med.* **169**, 431-445

34. Sternberg, E. M., Young, W. S., III, Bernardini, R., Calogero, A. E., Chrousos, G. P., Gold, P. W., and Wilder, R. L. (1989) A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to strep-tococcal cell wall-induced arthritis in Lewis rats. *Proc. Natl. Acad. Sci. USA* **86**, 4771-4775

35. Chelmicka-Schorr, E., Checinski, M., and Arnanson, B. G. W. (1988) Sympathectomy augments the severity of experimental allergic encephalomyelitis in rats. *Ann. N.Y. Acad. Sci. USA* **540**, 707-708

36. Hafler, D. A., and Weiner, H. L. (1989) MS: a CNS and systemic autoimmune disease. *Immunol. Today* **10**, 104-107

37. Feuer, C., Prentice, D. E., and Cammisuli, S. (1985) Chronic relapsing experimental allergic encephalomyelitis in the Lewis rat. *J. Neuroimmunol.* **10**, 159

38. Mokhtarian, F., McFarlin, D. E., and Raine, C. S. (1984) Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice. *Nature (London)* **309**, 356-358

39. McCarron, R. M., Fallis, R. J., and McFarlin, D. E. (1990) Alterations in T cell antigen specificity and class II restriction during the course of chronic relapsing experimental allergic encephalomyelitis. *J. Neuroimmunol.* **29**, 73-79

40. Williams, R. M., Lees, M. B., Cambi, F., and Macklin, W. B. (1982) Chronic experimental allergic encephalomyelitis induced in rabbits by bovine white matter proteolipid apoprotein. *J. Neuropathol. Exp. Neurol.* **41**, 508-521

41. Schluesener, H. J., Sobel, R. A., Linington, C., and Weiner, H. L. (1987) A monoclonal antibody against myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J. Immunol.* **139**, 4016-4021

42. Driscoll, B. F., Kies, M. W., and Alvord, E. C., Jr. (1974) Successful treatment of experimental allergic encephalomyelitis in guinea pigs with homologous myelin basic protein. *J. Immunol.* **112**, 392-396

43. Swierkosz, J. E., and Swanborg, R. H. (1975) Suppressor cell control of unresponsiveness to experimental allergic encephalomyelitis. *J. Immunol.* **115**, 631-635

44. Higgins, P. J., and Weiner, H. L. (1988) Suppression of experimental allergic encephalomyelitis by oral administration of myelin basic protein and its fragments. *J. Immunol.* **140**, 440-445

45. Bitar, D., and Whitacre, C. C. (1988) Suppression of experimental autoimmune encephalomyelitis by the oral administration of myelin basic protein. *Cell. Immunol.* **112**, 364-370

46. Brod, S. A., al-Sabbagh, A., Sobel, R. A., Hafler, D. A., and Weiner, H. L. (1991) Suppression of chronic relapsing experimental autoimmune encephalomyelitis by oral administration of myelin antigens. *Ann. Neurol.* **29**, 615-622

47. Sriram, S., Schwartz, G., and Steinman, L. (1983) Administration of myelin basic protein-coupled spleen cells prevents experimental allergic encephalomyelitis. *Cell. Immunol.* **75**, 378-382

48. Kennedy, M. K., Dal Canto, M. C., Trotter, J. L., and Miller, S. D. (1988) Specific immune regulation of chronic relapsing experimental allergic encephalomyelitis in mice. *J. Immunol.* **141**, 2986-2993

49. Kennedy, M. K., Tan, L.-J., Dal Canto, M. C., Tuohy, V. K., Lu, Z., Trotter, J. L., and Miller, S. D. (1990) Inhibition of murine relapsing experimental autoimmune encephalomyelitis by immune tolerance to proteolipid protein and its encephalitogenic peptides. *J. Immunol.* **144**, 909-915

50. Wells, H. G. (1911) Studies on the chemistry of anaphylaxis (III). Experiments with isolated proteins, especially those of the hen's egg. *J. Infect. Dis.* **9**, 147-171

51. Chase, M. W. (1946) Inhibition of experimental drug allergy by prior feeding of the sensitized agent. *Proc. Soc. Exp. Biol. Med.* **61**, 257-259

52. Mowat, A. M. (1987) The regulation of immune responses to dietary protein antigens. *Immunol. Today* **8**, 93-98

53. Mattingly, J. A. (1984) Immunologic suppression after oral administration of antigen. III. Activation of suppressor-inducer cells in the Peyer's patches. *Cell. Immunol.* **86**, 46-52

54. Mayer, L., and Shlien, R. (1987) Evidence for function of Ia molecules on gut epithelial cells in man. *J. Exp. Med.* **166**, 1471-1483

55. Nussenblatt, R. B., Caspi, R. R., Mahdi, R., Chan, C.-C., Roberge, F., Lider, O., and Weiner, H. L. (1989) Inhibition of S-antigen induced experimental autoimmune uveoretinitis by oral

induction of tolerance with S-antigen. *J. Immunol.* **144**, 1689–1695.

56. Thompson, H. S. G., and Staines, N. A. (1986) Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats. *Clin. Exp. Immunol.* **64**, 581–586

57. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W., and Thorbecke, G. L. (1986) Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl. Acad. Sci. USA* **83**, 7443–7446

58. Zang, Z. J., Lee, C. S. Y., Lider, O., and Weiner, H. L. (1990) Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. *J. Immunol.* **145**, 2489–2493

59. Miller, A., Lider, O., and Weiner, H. L. (1991) Antigen-driven bystander suppression following oral administration of antigens. *J. Exp. Med.* In press

60. Adorini, L., Harvey, M. A., Miller, A., and Sercarz, E. E. (1979) Fine specificity of regulatory T cells. II. Suppressor and helper T cells are induced by different regions of hen egg-white lysosome in a genetically non responder mouse strain. *J. Exp. Med.* **150**, 293–297

61. Swanborg, R. H. (1975) Antigen-induced inhibition of experimental allergic encephalitis. III. Localization of an inhibitory site distinct from the major encephalitogenic determinant of myelin basic protein. *J. Immunol.* **114**, 191–194

62. Teitelbaum, D., Aharoni, R., Arnon, R., and Sela, M. (1988) Specific inhibition of the T-cell response to myelin basic protein by the synthetic copolymer Cop 1. *Proc. Natl. Acad. Sci. USA* **85**, 9724–9728

63. Lando, Z., Teitelbaum, D., and Arnon, R. (1979) Effect of cyclophosphamide on suppressor cell activity in mice unresponsive to EAE. *J. Immunol.* **123**, 2156–2160

64. Bornstein, M. B., Miller, A., Slagle, S., Weitzman, M., Crystal, H., Drexler, E., Keilson, M., Merriam, A., Wassertheil-Smoller, S., Spada, V., Weiss, W., Arnon, R., Jacobsohn, I., Teitelbaum, D., and Sela, M. (1987) A pilot trial of Cop 1 in exacerbating-remitting multiple sclerosis. *N. Engl. J. Med.* **317**, 408–414

65. Ben-Nun, A., Wekerle, H., and Cohen, I. R. (1981) Vaccination against autoimmune encephalomyelitis using attenuated cells of a T lymphocyte line reactive against myelin basic protein. *Nature (London)* **292**, 60–61

66. Lider, O., Reshef, T., Beraud, E., Ben-Nun, A., and Cohen, I. R. (1988) Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science* **239**, 181–183

67. Acha-Orbea, H., Mitchell, D. J., Timmermann, L., Wraith, D. C., Tausch, G. S., Waldor, M. K., Zamvil, S. S., McDevitt, H. O., and Steinman. L. (1988) Limited heterogeneity of T cell encephalomyelitis allows specific immune intervention. *Cell* **54**, 263–273

68. Zaller, D. M., Osman, G., Kanagawa, O., and Hood, L. (1990) Prevention and treatment of murine experimental allergic encephalomyelitis with T cell receptor Vb-specific antibodies. *J. Exp. Med.* **171**, 1943–1955

69. Heber-Katz, E., and Acha-Orbea, H. (1989) The V-region disease hypothesis: evidence from autoimmune encephalomyelitis. *Immunol. Today* **10**, 164–169

70. Vandenbark, A. A., Hashim, G., and Offner, H. (1989) Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature (London)* **341**, 541–544

71. Howell, M. D., Winters, S. T., Olee, T., Powell, H. C., Carlo, D. J., and Brostoff, S. W. (1989) Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. *Science* **246**, 668–670

72. Wucherpfennig, K. W., Kohei, O., Endo, N., Seidman, J. G., Rosenweig, A., Weiner, H. L., and Hafler, D. A. (1990) Shared human T cell receptor Vb usage to immunodominant regions of myelin basic protein. *Science* **248**, 1016–1010

73. Hafler, D. A., Duby, A. D., Lee, S. J., Benjamin, D., Seidman, J. G., and Weiner, H. L. (1988) Oligoclonal T lymphocytes in the cerebrospinal fluid of patients with multiple sclerosis. *J. Exp. Med.* **167**, 1313–1322

74. Stamenkovic, I., Stegano, M., Wright, K. A., Krane, S. M., Amento, E. P., Colvin, R. B., Duquesnoy, R. J., and Kurnick, J. T. (1988) Clonal dominance among T-lymphocyte infiltrates in arthritis. *Proc. Natl. Acad. Sci. USA* **85**, 1179–1183

75. Weiner, H. L., Cohen, I. R., and Hafler, D. A. (1989) Immunologic effects following T cell vaccination in chronic progressive multiple sclerosis. *Neurology* (Suppl. 1) **39**, 172

76. Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L., and McDevitt, H. O. (1989) Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell* **59**, 247–255

77. Urban, J. L., Horvath, S. J., and Hood, L. (1989) Autoimmune T cells: immune recognition of normal and variant peptide-based therapy. *Cell* **59**, 257–271

78. Lamont, A. G., Sette, A., Fujinami, R., Colon, S. M., Miles, C., and Grey, H. M. (1990) Inhibition of experimental autoimmune encephalomyelitis induction in SJL/J mice by using a peptide with high affinity for IA^s molecules. *J. Immunol.* **145**, 1687–1693

79. Sriram, S., and Steinman, L. (1983) Anti I-A antibody suppresses active encephalomyelitis: treatment model for diseases linked to Ir genes. *J. Exp. Med.* **158**, 1362–1367

80. Boitard, C., Bendelac, A., Richard, M. F., Carnaud, C., and Bach, J. F. (1988) Prevention of diabetes in nonobese diabetic mice by anti-I-A monoclonal antibodies: transfer of protection by splenic T cells. *Proc. Natl. Acad. Sci. USA* **85**, 9719–9723

81. Perry, L. L., and Greene, M. I. (1982) Conversion of immunity to suppression by in vivo administration of I-A subregion-specific antibodies. *J. Exp. Med.* **156**, 480–491.

82. Brostoff, S. W., and Mason, D. W. (1984) Experimental allergic encephalomyelitis: successful treatment in vivo with a monoclonal antibody that recognizes T helper cells. *J. Immunol.* **133**, 1938–1942

83. Waldor, M. K., Sriram, S., Hardy, R., Herzenberg, L. A., Lanier, L., Lim, M., and Steinman, L. (1985) Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. *Science* **227**, 415–417

84. Sriram, S., and Roberts, C. A. (1986) Treatment of established chronic relapsing experimental allergic encephalomyelitis with anti-L3T4 antibodies. *J. Immunol.* **136**, 4464–4469

85. Schluesener, H. J., Sobel, R. A., and Weiner, H. L. (1988) Demyelinating experimental allergic encephalomyelitis (EAE) in the rat: treatment with a monoclonal antibody against activated T cells. *J. Neuroimmunol.* **18**, 341–351

86. Kelley, V. E., Gaulton, G. N., Hattori, M., Ikegami, H., Eisenbarth, G., and Strom, T. B. (1988) Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. *J. Immunol.* **140**, 59–61

87. Hafler, D. A., Fallis, R. J., Dawson, D. M., Schlossman, S. F., Reinherz, E. L., and Weiner, H. L. (1986) Immunologic responses of progressive multiple sclerosis patients treated with an anti-T-cell monoclonal antibody, anti-T12. *Neurology* **36**, 777–784

88. Hafler, D. A., Ritz, J., Schlossman, S. F., and Weiner, H. L. (1988) Anti-CD4 and anti-CD2 monoclonal antibody infusions in subjects with multiple sclerosis. *J. Immunol.* **141**, 131–138

89. Marx, J. (1990) Taming rouge immune reactions. *Science* **249**, 246–249

90. Ling, P. D., Warren, M. K., and Vogel, S. N. (1985) Antagonistic effect of interferon-beta on the interferon-gamma-induced expression of Ia antigen in murine macrophages. *J. Immunol.* **135**, 1857–1863

91. Hafler, D. A., and Weiner, H. L. (1987) In vivo labeling of blood T cells: rapid traffic into cerebrospinal fluid in multiple sclerosis. *Ann. Neurol.* **22**, 89–93

92. Lider, O., Baharav, E., Medori, Y. A., Miller, T., Naparstek, Y., Vlodavsky, I., and Cohen, I. R. (1989) Suppression of experimental autoimmune diseases and prolongation of allograft survival by treatment of animals with low doses of heparins. *J. Clin. Invest.* **83**, 752–756

93. Lussier, M. L., Weiner, H. L., Hafler, D. A., Dawson, D. M., Cohen, I. R., Stazzone, L., and Francis, G. (1988) Altering lymphocyte traffic: a new approach for the treatment of multiple sclerosis. *Neurology* **38** (Suppl. 1), 236

94. Weiner, H. L., and Hafler, D. A. (1988) Immunotherapy of multiple sclerosis. *Ann. Neurol.* **3**, 211–222
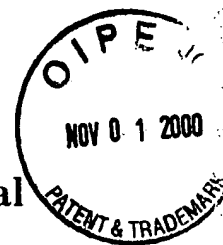
Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin

(diabetes/tolerance/autoimmunity/immunotherapy/insulin)

Z. JENNY ZHANG*, LAURIE DAVIDSON†, GEORGE EISENBARTH†, AND HOWARD L. WEINER*

*Brigham and Women's Hospital and †Joslin Diabetes Center, Harvard Medical School, Boston, MA 02115

ABSTRACT   Nonobese diabetic (NOD) mice spontaneously develop an autoimmune form of diabetes associated with insulitis. A number of immunomodulatory therapies have been investigated as a treatment for the disease process. Oral administration of the autoantigens myelin basic protein and collagen type II suppresses experimental models of encephalomyelitis and arthritis. We have now found that oral administration of insulin delays the onset and reduces the incidence of diabetes in NOD mice over a 1-year period in animals administered 1 mg of porcine insulin orally twice a week for 5 weeks and then weekly until 1 year of age. As expected, orally administered insulin had no metabolic effect on blood glucose levels. The severity of lymphocytic infiltration of pancreatic islets was also reduced by oral administration of insulin. Furthermore, splenic T cells from animals orally treated with insulin adoptively transfer protection against diabetes, demonstrating that oral insulin administration generates active cellular mechanisms that suppress disease. These results show that oral insulin affects diabetes and the pancreatic cellular inflammatory process in the NOD mouse and raise the possibility that oral administration of insulin or other pancreatic autoantigens may provide a new approach for the treatment of autoimmune diabetes.

Type I diabetes or insulin-dependent diabetes mellitus (IDDM) is thought to be an autoimmune disease in humans (1–3). The nonobese diabetic (NOD) mouse spontaneously develops IDDM that has many immunological and pathological similarities to human insulin-dependent diabetes. The autoimmune nature of the disease is suggested by the lymphocytic infiltration of the islets of Langerhans, which precedes the destruction of insulin-producing beta cells (4). As such, the NOD mouse has served as one of the primary models for IDDM and a model in which new approaches for immunotherapy have been investigated.

A variety of immunomodulatory treatments have been studied in the NOD mouse. In general, treatments that affect T-cell function or are immunosuppressive have been effective, such as neonatal thymectomy and in vivo treatment with anti-CD4 monoclonal antibody and cyclosporine A (5–7). A major impetus behind such studies has been to develop approaches that may be utilized to treat human IDDM. Clinical trials in humans have demonstrated that antigen nonspecific immunosuppression with drugs such as cyclosporine A and azathioprine can affect beta-cell destruction after diabetes onset, but such therapy is not curative and is associated with drug-related toxicities (8, 9). The ability to identify patient populations at risk for diabetes (10, 11) makes the development of disease-specific nontoxic forms of therapy that can be administered to prediabetics to prevent or reduce the incidence of diabetes a major therapeutic goal.

We have been investigating antigen-driven peripheral immune tolerance as a means to suppress autoimmune processes, using the oral route of antigen exposure. Orally administered antigen stimulates the immune system in a physiologic fashion and has long been recognized to produce systematic immunologic hyporesponsiveness or tolerance (12–14). We and others have found that oral administration of autoantigens suppresses animal models of autoimmunity including experimental autoimmune encephalomyelitis (EAE) (15–17), adjuvant- and collagen-induced arthritis (18–20), and experimental autoimmune uveitis (21). Oral tolerance as a means to treat diabetes is especially attractive because of its virtual lack of toxicity and its inherent clinical applicability. In addition, such therapy could be applicable to pancreatic islet transplantation. In the present report, we have found suppression of diabetes in the NOD mouse by oral administration of insulin.

MATERIALS AND METHODS

Animals. NOD mice were purchased from Taconic Farms, maintained in our animal facility, and fed regularly with Purina Mouse Chow 5015 or 5008. The animals studied in experiments in Table 1 and Fig. 4 were housed in a conventional room, and those studied in all other experiments were housed in a virus antibody-free (VAF) facility. Female NOD mice were used for all experiments except for recipients in adoptive transfer experiments.

Assessment of Diabetes. Mice were monitored for development of diabetes weekly by urinary glucose testing with test strips (Eli Lilly). Glycosuric mice were then bled to check for glycemia by using a glucose analyzer (Beckman). Diabetes was confirmed by hyperglycemia (>13.8 mM) for 2 consecutive weeks.

Antigens. Porcine monocomponent insulin was purchased from Novo Biolabs (Danbury, CT). Myelin basic protein (MBP) was prepared as described (15).

Oral Administration of Antigen. Insulin or MBP in phosphate-buffered saline (PBS; 1.7 mM KH_2PO_4/5 mM Na_2HPO_4/150 mM NaCl) was administered to mice orally through a syringe fitted with a ball-type feeding needle in a volume of 0.5 ml per mouse per feeding.

Histopathology. The animals were sacrificed by cervical dislocation, and the pancreases were taken and immediately frozen. Cryosections (3 or 4 sections per mouse) were fixed with acetone and double-stained with (i) biotinylated monoclonal anti-thy-1.2 antibody plus avidin–peroxidase conjugate and (ii) monoclonal anti-beta-cell antibody (A2B5) plus alkaline phosphatase-conjugated anti-mouse IgM. The degree of insulitis was scored blindly by two independent observers using a semiquantitative scale ranging from 0 to 4: 0, normal islet with no sign of T-cell infiltration; 1, focal peri-islet T-cell infiltration; 2, more extensive peri-islet infil-

Abbreviations: NOD, nonobese diabetic; IDDM, insulin-dependent diabetes mellitus; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; VAF, virus antibody-free.

Medical Sciences: Zhang *et al.*

Proc. Natl. Acad. Sci. USA 88 *(1991)*   10253

on but with lymphocytes less than one-third of the islet area; 3, intraislet T-cell infiltration in one-third to one-half of the islet area; 4, extensive intraislet inflammation involving more than half of the islet area.

Adoptive Transfer of Diabetes and T-Cell Depletion. The adoptive transfer experiments were carried out by the method of Wicker *et al.* (22) with slight modifications. Briefly, donor splenocytes were prepared from newly diabetic female animals (diagnosed within 14 days), resuspended in Hanks' balanced salt solution (HBSS), and injected i.v. through the retroorbital plexus (1×10^7 cells per recipient) to 7-week-old male NOD mice, which were irradiated with 770 R from a ^{137}Cs source 24 hr prior to the transfer. Five-million modulator cells from insulin-fed or control-fed animals were cotransferred with splenocytes from newly diabetic animals into male recipients. For T-cell depletion, splenocytes from insulin-fed animals were incubated with anti-thy-1.2 monoclonal antibody (diluted 1:200; from Accurate Chemicals, Westbury, NY) at a concentration of 2×10^7 cells per ml, at room temperature for 60 min, followed by an incubation with Low-Tox rabbit complement (1:15; Cedarlane Laboratories, Hornby, ON, Canada) for 30 min at 37°C. Control cells were treated with complement alone. Cells were washed three times with HBSS prior to transfer. Five-million anti-thy-1.2- or complement-treated cells were cotransferred.

RESULTS AND DISCUSSION

A number of autoantigens have been identified as potential target antigens of an autoimmune attack that leads to the development of diabetes. These include insulin, glutamic acid decarboxylase (GAD), carboxypeptidase H, insulin secretory granule proteins, and heat shock proteins (23–25). To test the effect of oral administration of insulin on the development of diabetes, female NOD mice at 5 weeks of age were fed PBS or 10 μg, 100 μg, or 1 mg of porcine insulin twice weekly for 5 weeks and then weekly until the animals reached 1 year of age. There was a marked delay in the onset and a decreased incidence of diabetes in animals fed 1 mg of porcine insulin (Table 1; Fig. 1, $P = 0.02$, Kaplan–Meier analysis) with a slight effect at 100 μg. Note that the incidence of diabetes in the control group was relatively low. This may be related to the frequent handling of the animals associated with feeding and to the housing of the animals for this experiment in a non-VAF facility. To test the effect of oral insulin in animals with a higher incidence of diabetes, a second experiment was conducted in a VAF facility. In addition, a group of animals was also fed 1 mg of MBP as a control antigen. A decreased incidence of diabetes following oral insulin was observed, although the overall incidence of diabetes was higher. Specifically, the incidence of diabetes in animals at 30 weeks was as follows: 13 of 30 fed PBS, 14 of

Table 1. Suppression of IDDM in NOD mice by oral administration of porcine insulin

Feeding treatment	Diabetes incidence, %		
	6 months	9 months	12 months
Control (PBS)	20.5	44.1	49.2
Insulin in PBS			
μg	16.7	23.8	37.3
0 μg	11.1	28.5	43.8
1 mg	0*	8.0*	26.4†

Five-week-old female NOD mice (27–30 per group) were fed with various dosages of porcine insulin in PBS (control group received PBS alone) twice weekly for 5 weeks and weekly until 1 year of age. Beginning at 12 weeks of age, the mice were examined weekly for
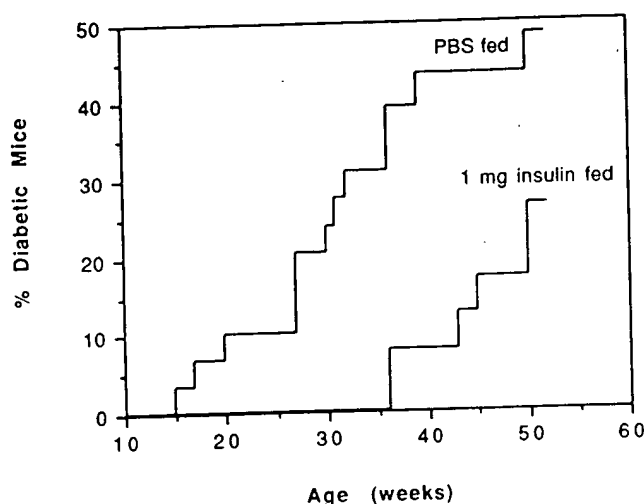

FIG. 1.   Effect of oral administration of porcine insulin on IDDM in female NOD mice. Life table analysis of the control group and the group fed 1 mg of insulin from Table 1 ($P = 0.02$, Kaplan–Meier analysis).

30 fed MBP, 13 of 29 fed 10 μg of insulin, 10 of 30 fed 100 μg of insulin, and 6 of 30 fed 1 mg of insulin ($P < 0.05$ for animals fed 1 mg of insulin vs. control and animals fed MBP).

It has been reported (26) that low doses of subcutaneous insulin may affect the onset of diabetes in NOD mice. Orally administered insulin is not metabolically active, presumably because it is degraded in the stomach. Degradation of proteins in the gastrointestinal tract does not affect oral tolerance and actually may facilitate orally induced tolerance by creating small protein fragments that are better able to interact with gut-associated lymphoid tissue (27). Nonetheless, to determine whether any metabolic effects could be discerned in animals being fed 1 mg of insulin, blood glucose levels were measured in 17-week-old animals. The average blood glucose prior to the weekly insulin feeding was 7.56 mM in animals fed PBS and 7.53 mM in animals fed 1 mg of insulin. Thirty minutes after feeding, the blood glucose in animals fed PBS
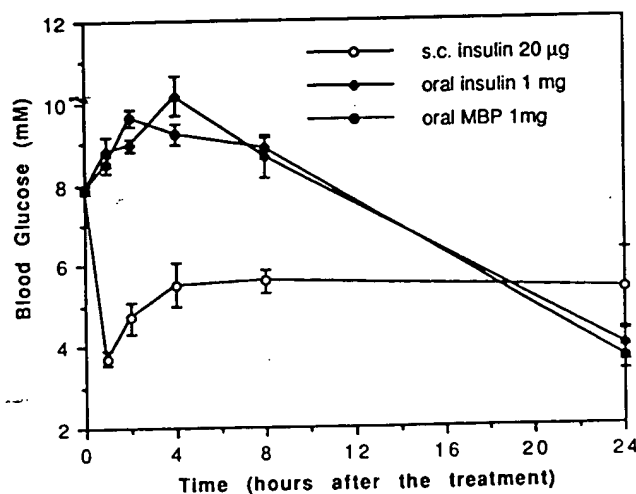
FIG. 2.   Effect of oral insulin on blood glucose. Seven-week-old female NOD mice (25 mice per group) were treated orally with 1 mg of porcine insulin or 1 mg of guinea pig MBP or were injected subcutaneously with 20 μg of porcine insulin. All mice were bled before treatment, and 5 mice from each group were bled again 1, 2, 4, 8, and 24 hr after treatment. Individual plasma samples were measured in duplicate for glucose levels by using a Beckman glucose
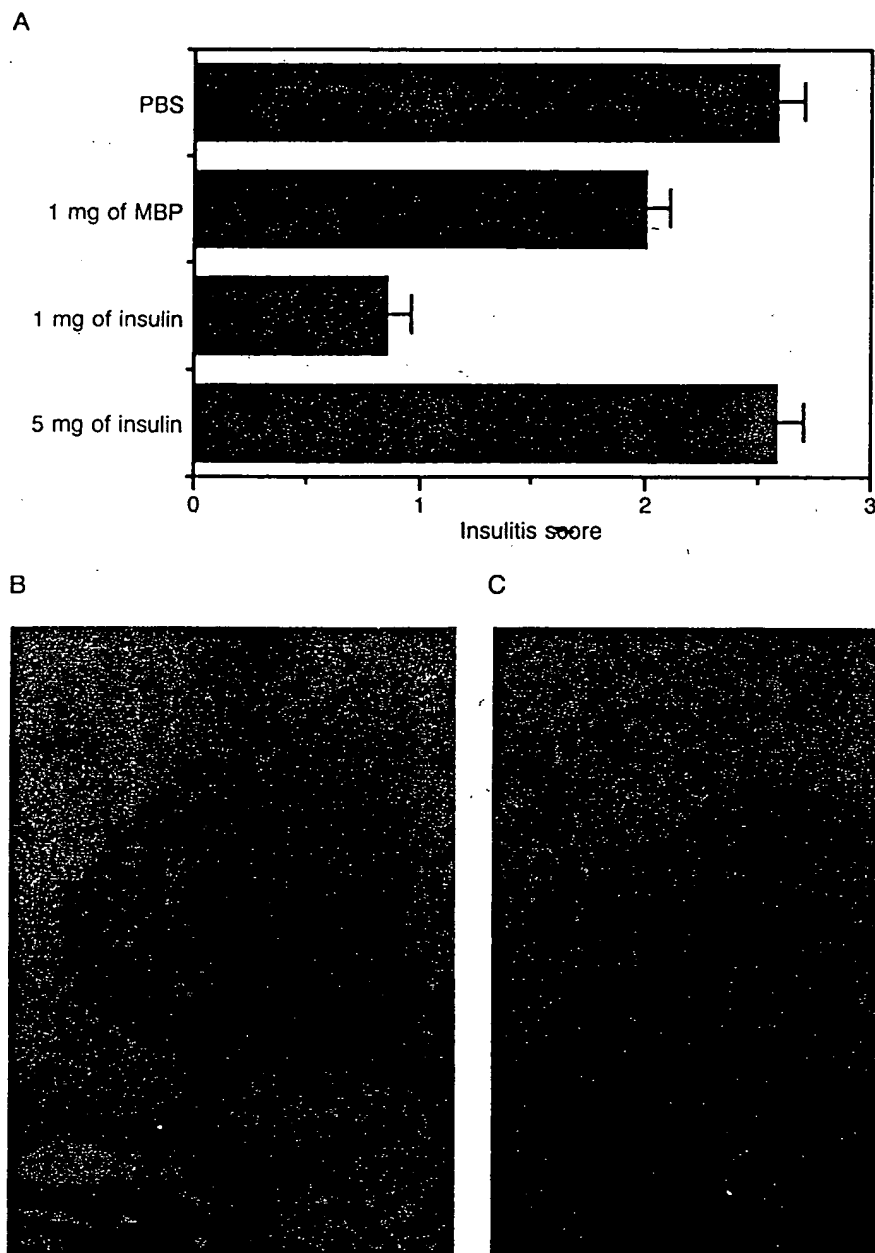
A

B   C

FIG. 3. Effect of feeding porcine insulin on insulitis in NOD mice. Five-week-old female NOD mice (8–10 mice per group) were fed PBS, 1 mg or 5 mg of insulin in PBS, or 1 mg of MBP in PBS twice weekly for 5 weeks. At 10 weeks of age, the animals were sacrificed, and pancreases were taken for histopathological examinations. Eight to 12 islets from each animal were scored. (A) Insulitis score. Data are expressed as the mean score of each group ± SEM ($P < 0.001$ for group fed 1 mg of insulin vs. group fed PBS or 1 mg of MBP). (B) Representative islet from control animal with pronounced lymphocyte infiltration (histopathologic score = 4). (C) Representative islet from animal fed 1 mg of insulin with minimal inflammation (histopathologic score = 1).

was 8.53 mM and in animals fed 1 mg of insulin was 8.63 mM. In an additional study, 7-week-old NOD mice were fed 1 mg of insulin or 1 mg of MBP. All animals were kept in a fasting state. Animals given 20 μg of subcutaneous insulin had an immediate drop in blood glucose (Fig. 2). In animals fed 1 mg of insulin or 1 mg of MBP, an increase in blood glucose was observed, perhaps related to the stress of gastric intubation, followed by a decrease in blood glucose 8 hr later as the animals were in a fasting state. Note that animals fed 1 mg of insulin for 1 year responded normally to subcutaneous insulin (data not shown). These results show that oral insulin has no metabolic effect on blood glucose either acutely or chronically.

To determine whether feeding insulin affected lymphocytic infiltration of pancreatic islets, animals in a separate series of experiments were fed 1 mg of insulin twice weekly for 5 weeks and were sacrificed at 10 weeks of age and examined for insulitis. There was a marked reduction of insulitis in animals fed 1 mg of insulin vs. those fed 1 mg of MBP (Fig. 3; 0.85 ± 0.1 vs. 1.99 ± 0.1; $P < 0.01$). Note that feeding 5 mg of insulin did not affect insulitis. A similar dose–response effect has been observed with oral tolerization to collagen in

animal models of adjuvant and collagen-induced arthritis in which the suppressive effect of oral collagen was lost with increased doses (18–20). We also have observed a loss of suppression of EAE in the SJL mouse by orally administered MBP with increasing doses (28).

The majority of studies on the mechanism of oral tolerance report that active cellular suppression occurs (14). We have adoptively transferred disease protection with lymphocytes from fed animals in both the EAE and adjuvant arthritis models (16–18). To investigate whether active cellular mechanisms were associated with suppression of diabetes in the NOD mouse after oral administration of insulin, an accelerated diabetes model was utilized, in which diabetes is accelerated in young NOD mice by adoptive transfer of splenocytes from diabetic NOD donors; this provides a sensitive and more rapid assay for investigating immunomodulation of disease. Spleen cells from animals fed 1 mg of insulin, PBS, or 1 mg of MBP 5 times over 2 weeks were cotransferred with spleen cells from diabetic animals. Accelerated diabetes in NOD mice was suppressed by splenocytes from insulin-fed but not PBS- or MBP-fed animals (Fig. 4; $P < 0.01$, "logrank" test for all groups).
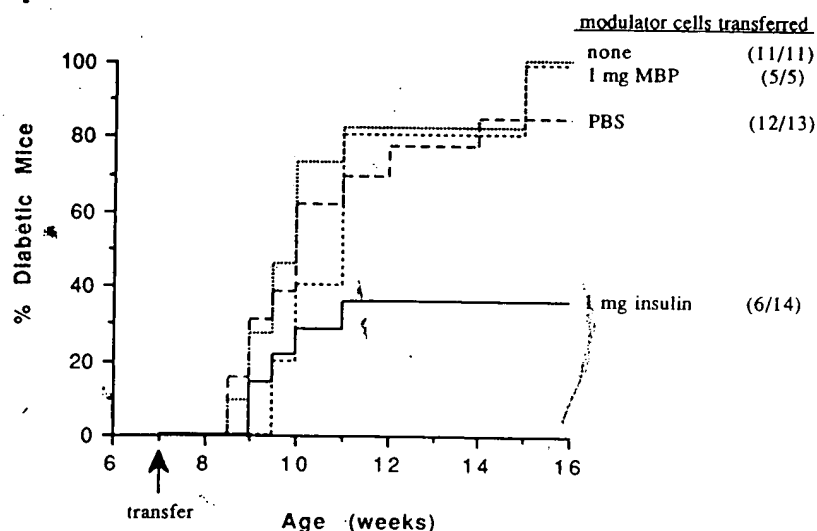
FIG. 4. Suppression of adoptively transferred diabetes by splenocytes from female NOD mice fed insulin. Modulator cells were freshly obtained from 6-week-old female NOD mice that had been fed 1 mg of insulin, 1 mg of MBP, or PBS five times over the previous 2 weeks. Ten million splenocytes isolated from female diabetic NOD mice were cotransferred with 5 million modulator cells from fed animals to 7-week-old syngeneic male recipients that had been irradiated with 770 R 24 hr earlier. The onset of diabetes in the recipients was checked twice weekly by assaying for glycosuria and confirmed by presence of hyperglycemia (>13.8 mM). $P = 0.037$ (logrank test) for all groups; $P = 0.021$ for animals fed insulin vs. those fed PBS.

To determine whether the suppression was T cell dependent, T cells were depleted from splenocytes of insulin-fed animals prior to adoptive transfer. Eight weeks after transfer, the incidence of accelerated diabetes in animals receiving no modulators was 10/11; in animals receiving complement-alone-treated modulators from insulin-fed animals was 2/10, and in animals receiving T cell-depleted modulators from insulin-fed animals was 9/10 ($P = 0.02$). Others have reported suppression of accelerated disease with transfer of 20×10^6 spleen cells from nondiabetic 8-week-old animals (29). We not observe protection by spleen cells from control animals with 5×10^6 cells transferred.

The effects we observed in the NOD mouse are not related to nonspecific suppressive effects of orally administered insulin as oral administration of 1 mg of insulin had no effect on the development of EAE in the SJL mouse or on cellular proliferative response to concanavalin A or lipopolysaccharide. (data not shown). In other studies of oral tolerance in autoimmune models, we also found disease protection to be antigen and disease specific. Thus, the antigens we have used for oral tolerization, MBP, collagen type II, and S antigen suppress EAE, adjuvant arthritis, and experimental uveitis, respectively, without affecting the other diseases. Species-specific autoantigens are not a requisite to induce oral tolerance as we have found suppression of EAE in the Lewis rat with bovine MBP.

Although we have shown suppression of diabetes and insulitis in the NOD mouse by oral administration of insulin, the role of autoimmunity to insulin in the development of diabetes in the NOD mouse and in man remains to be defined. Anti-insulin antibodies are found in both NOD mice and patients with type I diabetes (30). In patients, anti-insulin autoantibodies can be found prior to the onset of insulin therapy, are HLA-DR4-associated, and are correlated with the rate of disease progression (31, 32). Cellular reactivity to insulin occurs in man and has been reported to be of increased frequency in prediabetic individuals (33). Cellular immunity to insulin has not been extensively studied in the NOD mouse, and in initial experiments we have not found cellular immune responses to insulin as measured by thymidine incorporation he spleen or lymph nodes of NOD mice, though further tigations are required in this area using more sensitive ys and studying cells isolated from the pancreas.

Adoptive transfer experiments demonstrate that transferable active suppression of diabetes in the NOD mouse by splenic T cells is generated by oral administration of insulin. Recent studies from our laboratory suggest that the T cells that adoptively transfer suppression of experimental autoimmune encephalomyelitis following oral administration of

MBP are triggered in an antigen-specific fashion but mediate their effect by the release of the antigen-nonspecific suppressor cytokine transforming growth factor β (TGF-β) in close proximity to effector cells (34). We have termed this mechanism "antigen-driven bystander suppression" (35). Thus, it is possible that insulin is not a pathogenic autoantigen in the NOD mouse but that the regulatory cells generated in the gut by feeding insulin migrate to the pancreas and are triggered by insulin to release TGF-β, which down-regulates the local inflammatory processes in the pancreas. Further investigations are required to determine whether oral administration of insulin affects diabetes in the NOD mouse by suppression of anti-insulin autoimmunity or by the aforementioned antigen-driven bystander suppression mechanism.

It remains to be determined whether oral administration of other islet cell-specific antigens such as glutamic acid decarboxylase, carboxypeptidase H, heat shock proteins, or secretory granule proteins can also suppress diabetes in the NOD mouse. For application to human disease states, we have found that oral administration of autoantigens suppresses both established EAE and adjuvant arthritis, demonstrating the ability to effect an ongoing immune response (18, 36).

Although our data clearly demonstrate amelioration of diabetes in the NOD mouse by oral administration of insulin, protection is not complete. We have observed that adjuvants such as lipopolysaccharide, when given orally, enhance the protective effects of oral tolerance to MBP in the EAE model (37). In addition, after week 10 of the NOD mouse, insulin was administered once per week. Thus, the use of tolerogenic adjuvants to enhance suppression, or more frequent dosing schedules, may lead to more complete prevention of diabetes. Additionally, it may be that oral administration of more than one or a different pancreatic target antigen will further enhance protection.

One of the primary goals for the immunotherapy of autoimmune diseases is to find nontoxic antigen-specific therapies that can be administered early in the course of the disease. Our results in the NOD mouse model of diabetes raise the possibility that orally administered insulin and/or other pancreatic antigens could provide a new approach for the prevention and treatment of autoimmune diabetes in man.

1. Castano, L. & Eisenbarth, G. S. (1990) *Annu. Rev. Immunol.*

2. Rossini, A., Mordes, J. P. & Greiner, D. L. (1989) *Curr. Opinion Immunol.* **2**, 598–603.
3. Acha-Orbea, H. & McDevitt, H. O. (1990) *Curr. Top. Microbiol. Immunol.* **156**, 103–119.
4. Leiter, E. H., Serreze, D. V. & Prochaz, K. A. M. (1990) *Immunol. Today* **11**, 147–149.
5. Ogawa, M., Maruyama, T., Hasegawa, T., Kanaya, T., Kobayash, F., Tochino, Y. & Uda, H. (1985) *Biomed. Res.* **6**, 103–105.
6. Mori, Y., Suko, M., Okudiara, H., Matsuba, I., Tsuruoka, S., Sasaki, A., Yokoyama, H., Tanase, T., Shida, T., Nishimura, M., Terada, E. & Ikeda, Y. (1986) *Diabetologia* **29**, 244–247.
7. Shizuru, A., Edwards-Taylor, C., Banks, B. A., Gregory, A. K. & Fathman, C. G. (1988) *Science* **240**, 659–662.
8. Silverstein, J., Maclaren, N., Riley, W., Spillar, R., Radjenovic, D. & Johnson, S. (1988) *N. Engl. J. Med.* **319**, 599–604.
9. Bougneres, P. F., Landais, P., Boisson, C., Carel, J. C., Frament, N., Boitard, C., Chaussain, J. L. & Bach, J. F. (1990) *Diabetes* **39**, 1264–1272.
10. Ziegler, A. G., Herskowitz, R. D., Jackson, R. A., Soeldner, J. S. & Eisenbarth, G. S. (1990) *Diabetes Care* **13**, 762–775.
11. Bonifacio, E., Bingley, P. J., Shattock, M., Dean, B. M., Dunger, D., Gale, E. A. M. & Bottazzo, G. F. (1990) *Lancet* **335**, 147–149.
12. Wells, H. G. (1911) *J. Infect. Dis.* **9**, 147–171.
13. Chase, M. (1946) *Proc. Soc. Exp. Biol. Med.* **61**, 257–259.
14. Mowat, A. M. (1987) *Immunol. Today* **8**, 93–98.
15. Higgins, P. J. & Weiner, H. L. (1988) *J. Immunol.* **140**, 440–445.
16. Lider, O., Santos, L. M. B., Lee, C. S. Y., Higgins, P. J. & Weiner, H. L. (1989) *J. Immunol.* **142**, 748–752.
17. Bitar, D. M. & Whitacre, C. C. (1988) *Cell. Immunol.* **112**, 364–370.
18. Zhang, J. Z., Lee, C. S. Y., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **145**, 2489–2493.
19. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, F. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7443–7446.
20. Thompson, H. S. G. & Staines, N. A. (1986) *Clin. Exp. Immunol.* **64**, 581–586.
21. Nussenblatt, R. B., Caspi, R., Mahdi, R., Chan, C., Roberge, F., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **144**, 1689–1695.
22. Wicker, L. S., Miller, B. J. & Mullen, Y. (1986) *Diabetes* **35**, 855–860.
23. Baekkeskov, S., Aanstoot, H., Christgau, S., Reetz, A., Solimena, M., Cascalho, M., Folli, F., Richter-Olesen, H. & Camilli, P. (1990) *Nature (London)* **347**, 151–156.
24. Roep, B. O., Arden, S. D., de Vries, R. R. P. & Hutton, J. C. (1990) *Nature (London)* **345**, 632–634.
25. Elias, D., Markovits, D., Reshef, T., van der Zee, R. & Cohen, I. R. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1576–1580.
26. Atkinson, M. A., Maclaren, N. K. & Luchetta, R. (1990) *Diabetes* **39**, 933–937.
27. Michael, J. G. (1989) *Immunol. Invest.* **18**, 1049–1054.
28. Al-Sabbagh, A. & Weiner, H. L. (1991) *Neurology* **41**, 318 (abstr.).
29. Boitard, C., Yasunami, R., Dardenne, M. & Bach, J. F. (1989) *J. Exp. Med.* **169**, 1669–1680.
30. Ziegler, A. G., Vardi, P., Ricker, A. T., Hattori, M., Soeldner, J. S. & Eisenbarth, G. S. (1989) *Diabetes* **38**, 358–363.
31. Ziegler, R., Alper, C. A., Awdeh, Z. L., Castano, L., Brink, S. J., Soeldner, J. S., Jackson, R. & Eisenbarth, G. S. (1991) *Diabetes* **40**, 709–714.
32. Palmer, J. P., Asplin, C. M., Clemons, P., Lyon, K., Iatpati, O., Raghu, P. & Paquette, T. L. (1983) *Science* **222**, 1337–1339.
33. Keller, R. J. (1990) *J. Autoimmun.* **3**, 321–327.
34. Miller, A., Lider, O., Roberts, A. B., Sporn, M. B. & Weiner, H. L. (1991) *Proc. Natl. Acad. Sci. USA*, in press.
35. Miller, A. M., Lider, O. & Weiner, H. L. (1991) *J. Exp. Med.* **174**, 791–798.
36. Brod, S. A., Al-Sabbagh, A., Sobel, R. A., Hafler, D. A. & Weiner, H. L. (1991) *Ann. Neurol.* **29**, 615–622.
37. Khoury, S. J., Lider, O., Al-Sabbagh, A. & Weiner, H. L. (1990) *Cell. Immunol.* **131**, 302–310.

TABLE OF CONTENTS

CURRICULUM·VITAE

Matthias G. von Herrath

Personal Data:

Date of birth: 30 June, 1963
Place of birth: Berlin, Germany
Nationality: German, US permanent resident
Marital Status: Married
Social Security: 607-58-9399

Education:

Primary School in Berlin, 1969
Gymnasium (Classical Secondary School), 1973-1982
Freiburg Medical School (University), 1982-1988

MD-degree (Final medical examination) 1988
Doctoral (Ph.D.) Degree (Thesis in Microbiology) rated "summa cum laude"1988
Full medical license (Approbation) 1990

First part Educational Commission for Foreign Medical Graduates examination, 1986
Second part ECFMG examination, 1992
California Medical Boards (FLEX/USMLE), 1993

Professional Experience:

Doctoral Ph.D. thesis, Freiburg University, laboratory of Professor Holzer
    (Microbiology), 1984-1986; finished "summa cum laude", 1988
Research in Freiburg University Cardiology Department, (Professor Just), 1984-1987
Course in gene technology, NIH, Molecular Biology Laboratory, 1984
Gene technology research program, NIH (mentor: Professor Freese), 1986
Internship, Freiburg Medical Center, Internal Medicine (Professor Peter), Immunology, 1988-
    1989
Residency (Internal Medicine), Intensive Care Unit, Diakonic Hospital, Freiburg, (Professor
    Arnold), 1/1989-01/1991
Postdoctoral Fellow, Department of Neuropharmacology, The Scripps Research Institute, La
    Jolla, California, (Dr. M.B.A. Oldstone), 2/1991-2/1995
Senior Research Associate (Instructor) with Dr. M.B.A. Oldstone, The Scripps Research
    Institute, Department of Neuropharmacology, La Jolla, California, 2/1995-5/1996
Assistant Professor, The Scripps Research Institute, 6/1996 – 5/1999
Associate Professor, The Scripps Research Institute, 6/1999 - present

Current grant support

JDF Career Development Award, July 1996, for 4 years   75,000$/year
   (DNA vaccination to treat IDDM/mechanism of β-cell destruction)
NIH (NIDDK) R29 grant June 1996, for 5 years   72,000$/year
   (Oral tolerance to treat IDDM, cytokines in diabetes pathogenesis)
NIH (NIAID) R01 grant October1998, for 3 years   163,000$/year
   (Regulatory lymphocytes to treat IDDM, cytokine signaling pathways
   in IDDM, costimulation blockade to treat IDDM)
NIH (AG) Project on Program project grant, December 1998, for 5 years   89,000$/year
   (effect of age on cytokines and autoreactive cells in autoimmune disease)

Total: 399,000$/year

Awards and Fellowships

DAAD Fellowship - Gene technology - 1986
DFG Postdoctoral Fellowship - 1991-1993
JDF (Juvenile Diabetes Foundation) Fellowship Award - 1993-1995
ASV Travel fellowship for Int. Congress of Virology 8/96
AAI Travel fellowship for Int. Congress of Immunology 11/98

Individuals trained and recruited:

Independently:

1998-present: Urs Christen, Ph.D., Postdoctoral Fellow, supported by Swiss Nat.
         Foundation
1998-present: Angelika Jahreis, Postdoctoral Fellow, supported by German
         Leopoldina Fellowship
10-12/1998:   Mette Ejrnes, Ph.D. Student from the Hagedorn Res. Inst.
         Denmark, supported by NovoNordisk Stipend.
1-8/1999   :   Sonja Seewaldt, Ph.D. Student from the European Nat. University,
         Strassbourg, supported by DGFI and DAAD fellowships.
9/1999-8/2000:Ursula Möhrle, Student from the University Freiburg Medical Center, Freiburg,
         Germany, supported by the Carl Duisberg Foundation.

Jointly with Michael Oldstone:

1996-present: Dirk Homann, MD, Postdoctoral Fellow, supported by DAAD and
         SDV Stipends
1997-present: Andreas Holz, Ph.D., Postdoctoral Fellow, supported by Swiss Nat.
         Foundation

Professional Memberships

American Society of Virology
American Society of Immunology
International Diabetes Society
Juvenile Diabetes Foundation International
American Diabetes Association
World Affairs Council, San Diego

Study Sections:

Juvenile Diabetes Foundation, Study Section, permanent member 1999-2001
NCRR Comparative Medicine, Ad Hoc,   VA Grant Reviews
NIH ad hoc reviews for the NIAID and NIEHS

Reviews for journals:

Nature Medicine
Journal of Immunology
Journal of Virology
Virology
Immunology Today
Journal of Clinical Investigation
Hepatology
Journal of Autoimmunity
European Journal of Immunology
Archives of Virology
European Journal of Immunology

Invited Major Presentations:

2/1994 - Seminar, Department of Experimental Pathology, University of Zurich, Switzerland
Host: Professor Zinkernagel

2/1994 - Faculty Seminar, Max Planck Institute for Immunobiology, Freiburg, FRG
Host: Professor Eichman

2/1995 - Faculty Seminar, Dept. of Experimental Pathology, University of Zurich,
Host: Professor Zinkernagel

2/1995 - Faculty Seminar, Max Planck Institute for Immunobiology, Freiburg, FRG
Host: Professor Hartmut Peter

6/1995 - Invited Speaker, FASEB Summer Conference on Autoimmunity in Saxton River, Vermont, USA

3/1996 - Invited Speaker, International Royal Society of Medicine (London) "Molecular Mimicry" Meeting.   Host: Professor Harold Baum

6/1996 - Group Lecture, Department of Microbiology, Emory University Atlanta, USA
Host: Prof. R. Ahmed

6/1996 - Group Lecture, Department of Immunology, Yale University New Haven, USA
Host: Dr. R. Flavell

**8/1996 - Invited Speaker, University of Montreal, Canada,
Host: Dr. Trevor Owens**

**12/1996 - Faculty Seminar Series, Department of Experimental Pathology, Zurich,
Host: Prof. Zinkernagel**

12/1996 - Invited Plenary Speaker, International Immunology of Diabetes Congress, Canberra, Australia. Host: Professor Kevin Lafferty

12/1996 - Invited Plenary Speaker, British Society of Immunology Congress in Harrogate, UK. Host: Dr. Anne Cooke, Dr. Diego Vergani

3/1997 - Invited Plenary Speaker, German Virology Congress, Hamburg, FRG

Host: Professor Lehman-Grube

4/1997 - **Invited Speaker; Barbara Davis Center for Childhood Diabetes, Denver, CO,**
Host: Dr. Ron Gill

7/1997 - Invited <u>Plenary Speaker,</u> Lessons from Animal Diabetes (LAD) Workshop, Copenhagen

3/1998 - **Invited Speaker, ThymOz Conference, Heron Island, Australia,**
Host: Dr. R. Boyd

6/1998 - Invited Speaker, Immunology Seminar Series, Univ. of California, San Francisco,
Host: Prof. Steinunn Baekkeskov

6/1998 - Invited Speaker, Main Immunology Seminar Series, University of Cleveland,
OH, USA
Host: Prof. Paul Lehmann, Thomas Forsthuber

9/1998 - **Invited <u>Plenary Speaker</u>, German Immunology Congress, Freiburg, FRG**

10/1998 - **Invited Speaker, Symposium on Basic Aspects of Immunology by USCHS, Washington DC**

11/1998 - **Invited <u>Plenary Speaker</u>, International Congress of Immunology, New Delhi, India**

1/1999 - **Invited <u>Plenary Speaker</u>, Keystone Conference on Mucosal Immunology, Santa Fe, NM**

3/1999 - **Invited Speaker, 3rd International Congress of Autoimmunity, Tel Aviv, Israel**

8/1999 - **Invited Speaker, International Multiple Sxlerosis Workshop, Brighton, England**

9/1999 - **Invited Speaker, Chilean Society of Immunology Conference, Santiago, Chile**

9/1999 - **Invited Speaker, AASLD Conference on Autoimune Hepatitis, Atlanta, GA**

9/1999 - **Invited Speaker, Pathogenic and Regulatory Cell in Demyelinating Diseases Conference, Rome, Italy**

<u>Chaired Scientific Sessions and Workshops</u>:

12/1996 - Workshop Chair, International Immunology of Diabetes Congress, Canberra, Australia

4/1997 - **Co-Chair, Keystone Conference on Tolerance and Autoimmunity, Keystone, CO, USA**
Host: Polly Matzinger

6/1998 - **Invited Chair,** American Society of Virology Congress, Vancouver, B.C.,
Canada
Host: Sue Moyer

<u>Other Invited Presentations</u>:

2/1995 - Interdisciplinary Lecture, CNRS, Toulouse, France
Host: Dr. Jean E. Gairin

2/1995 - Group Lecture, NovoNordisk and University of Copenhagen, Denmark.
Host: Dr. Thomas Dyrberg

6/1995 - Group Lecture, Dept. of Cancer Biology, Harvard Medical School, Boston,
MA, USA
Host: Professor Laurie Glimcher

10/1995 - Seminar, Immunology Special Interest Group, Lab. Of Cell. and Mol.
Immunology, NIH, Washington DC, USA.

3/1996 - Group Lecture, NovoNordisk and Univ. of Copenhagen, Denmark.
Host: Dr. Thomas Dyrberg

3/1996 - Faculty Seminar, INSERM Paris, Hopital Necker,
Host: Prof. J.F. Bach

International References

Professor Jacques F.A.P. Miller
Director, Thymus Biology Unit
The Walter and Eliza Hall Institute
PO Royal Melbourne Hospital
Melbourne, VIC 3050
AUSTRALIA
Ph: 03 9 345 2555
Fax: 03 9 347 0852

Professor Laurie Glimcher
Chairman, Department of Immunology &
Infectious Diseases
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115
Ph: 617 432 0622
Fax: 617 432 0084
Lglimche@hsph.harvard.edu

Professor Marshall Horwitz
Chairman, Department of Microbiology
Albert Einstein College of Medicine
New York
Ph: 718-430-2230
Fax: 718-430-8702
horwitz@aecom.yu.edu

Professor Kevin J. Lafferty
Director, John Curtin School of Med. Research
Australian National University
Mills Road, GPO Box 334
Anu Acton, ACT 0200
AUSTRALIA
Ph: 616 249 2597
Fax: 616 249 2337
Director@jcsmr.anu.edu.au

Professor Polly Matzinger
Director, NIAID
National Institutes of Health
Bldg 4 Rm 111
Bethesda, MD 20894
Ph: 301 496 6440
Fax: 301 496 0877
pcm@helix.nih.gov

Professor Richard Flavell
Chairman, Department of Immunobiology
Yale University Medical School
310 Cedar St. FMB 412, HHMI Box 208011
New Haven, CT 06520-8011
Ph: 203 787 2216
Fax: 203 785 7561
richard.flavell@quickmail.yale.edu

Professor Jean-Francois Bach
Director, INSERM Unite 25
Necker Hospital
161, rue de Sevres
Paris cedex 15, 75743
France
Ph: 33 1 44 49 53 71
Fax: 33 1 43 06 23 88

Professor Hans-Hartmut Peter
Abt. Rheumatologie und Klinische
Immunologie/Med. Univ.Klinik
Hugstetterstr. 55
D-70106 Freiburg
Ph: 49 761 270 3448
Fax: 49 761 270 3446
Peter@mm61.ukl.uni-freiburg.de

Professor Hugh O. McDevitt
Chairman, Department of Immunology and
Rheumatology
Stanford University School of Medicine
Fairchild Bldg, D345
Palo Alto, CA 94305-5402
Ph: 415 723 5893
Fax: 415 723 9180
mk.mcd@forsythe.stanford.edu

Professor Rolf Zinkernagel
Dept. of Pathology
Univ. of Zürich
Schmelzbergstr. 55, Zürich, Switzerland
Ph: 011-41-255-4121
Fax: 011-41-255-4420

References at The Scripps Research Institute

Publications:

Manuscripts in preparation:

Jahreis, A., T. Wolfe and **von Herrath, M.G.** Therapy with anti-CD40L prevents IDDM by inducing anergy in memory autoreactive lymphocytes.

von Herrath, M.G., T. Dyrberg and J. Petersen. Coupling to CTB enhances oral tolerance – a useful predictive system.

von Herrath, M.G. and M. Oldstone. How multiple infections with related and unrelated viruses can enhance or abrogate autoimmune diabetes.

Holz, A., M.B.A. Oldstone and **M.G. von Herrath**. The role of B-lymphocytes in IDDM, for J. of Immunology.

Submitted articles:

Bot, A., Coon. B., and **von Herrath, M.G.** Overexpression of IL-4 in the lung interferes with priming and recruitment of influenza specific memory CTL. *Virology, in press, 2000*

Seewaldt, S., Thomas, H., Ejneas, M., Wolfe, T., Christen, U., Rodrigo, E., Coon, B., Michelsen, B., Kay, T.W.H. and **M. von Herrath**. Virus-induced autoimmune diabetes: Most β-cells die through inflammatory cytokines and not perforin from autoreactive (anti-viral) CTL. Submitted, 11/1999.

von Herrath, M.G., Berger, D., Wolfe, T., Hoimann, D., Sette, A., and M. Oldstone Vaccination to treat persistent infection. *Virology in press, 2000*

Original peer-reviewed first or last author articles:

Homann, D., A. Holz, A. Bot, B. Coon, T. Wolfe, J. Petersen, T.P. Dyrberg, M.J. Grusby, and **M.G. von Herrath**. Autoreactive CD4+ T cells protect from autoimmune diabetes via bystander suppression using the IL-4/Stat6 pathway. Immunity 11:463-472, 1999.

Holz, A., A. Bot, B. Coon, T. Wolfe, M.J. Grusby and **M.G. von Herrath**. Disruption of the STAT4 signaling pathway protects from autoimmune diabetes while retaining antiviral immune competence. J. Immunol., 163:5374-5382, 1999.

Coon, B., Ling-Ling, A., J.L. Whitton and **M.G. von Herrath**. DNA vaccination with self antigens prevents IDDM J. Clin. Invest. 104:189-194, 1999.

Homann, D., T. Dyrberg, J. Petersen, M.B.A. Oldstone and **M.G. Von Herrath**. Insulin in oral immune "tolerance": A one-amino acid change in the B chain makes the difference.. J. Immunol., 163: 1833-38, 1999.

von Herrath, M.G., Bryan Coon, Dirk Homann, Tom Wolfe and Luca G. Guidotti. Thymic tolerance to only one viral protein reduces lymphocytic choriomeningitis virus-induced immunopathology and increases survival in perforin deficient mice, J. Virol. , 73: 5918-25, 1999.

von Herrath, M.G., B. Coon, H. Lewicki, H. Mazarguil, J.E. Gairin, and M.B.A. Oldstone. In vivo treatment with a MHC class I restricted blocking peptide can prevent virus-induced autoimmune diabetes. J. Immunol., 161: 5087-99, 1998.

von Herrath, M.G., and A. Holz. Pathological changes in the islet milieu precede infiltration of islets and destruction of β-cells by autoreactive lymphocytes in a transgenic model of virus-induced IDDM. J. Autoimmunity 10:231-38, 1997.

von Herrath, M.G., D. Homann. Treatment of virus-induced IDDM by oral administration of insulin: Study on the mechanism by which oral antigens can abrogate autoimmunity Endocrinol. and Diabetes 105:24-25, 1997.

von Herrath, M.G., B. Coon and M.B.A. Oldstone. Low-affinity cytotoxic T-lymphocytes require γ-

interferon to clear an acute viral infection. Virology, 299:349-59, 1997.

von Herrath, M.G., S. Efrat, M.B.A. Oldstone and M.S. Horwitz. Expression of adenoviral E3 transgenes in β-cells prevents autoimmune diabetes. PNAS, 94:9808-13, 1997.

von Herrath, M.G., and M.B.A. Oldstone. Interferon-γ is essential for destruction of β-cells and development of IDDM. J. Exp. Med., 185:531-9, 1997.

von Herrath, M.G., T. Dyrberg and M.B.A. Oldstone. Oral insulin treatment suppresses virus-induced antigen specific destruction of beta-cells and prevents autoimmune diabetes in transgenic mice. J. Clin. Inv. 98:1324-1331, 1996.

von Herrath, M.G., M. Yokoyama, M.B.A. Oldstone and J.L. Whitton. CD4-deficient mice have reduced levels of memory CTL following immunization, and show diminished resistance to subsequent virus challenge. J. Virol., 70:1072-79, 1996.

von Herrath, M.G., S. Guerder, H. Lewicki, R. Flavell and M.B.A. Oldstone. Coexpression of B7.1 and Viral ("self") transgenes in pancreatic β-cells breaks peripheral tolerance: Spontaneous induction of autoimmune disease. Immunity, 3:727-738, 1995.

von Herrath, M.G., M.B.A. Oldstone and H. Fox. SIV-specific cytotoxic T-lymphocytes in cerebrospinal fluid and brains of SIV infected rhesus macaques. J. Immunol. 154:5582-5589, 1995.

von Herrath, M.G., J. Allison, J.F.A.P Miller and M.B.A. Oldstone. Focal expression of interleukin-2 does not break unresponsiveness to "self" (viral) antigen expressed in β cells but enhances development of autoimmune disease (diabetes) after initiation of an anti-self immune response. J. Clin. Invest. 95:477-485, 1995.

von Herrath, M.G., J. Dockter, M. Nerenberg, J. Gairin and M.B.A. Oldstone. Thymic selection and adaptability of CTL responses in transgenic mice expressing a viral protein in the thymus. J. Exp. Med., 180:1901-1910, 1994.

von Herrath, M.G., J. Dockter and M.B.A. Oldstone. How a virus induces a rapid or slow onset insulin-dependent diabetes mellitus in a transgenic model. Immunity, 1:231-242, 1994.

Review articles:

von Herrath, M.G. Strengths and pitfalls in Immunotherapy of IDDM: Novel insights from the RIP-LCMV transgenic mouse model of virus-induced diabetes. Tranpl. Proc. 30(8):4115, 1998.

von Herrath, M.G. Bystander suppression induced by oral tolerance. Research in Immunology, 148:541-554,1997.

von Herrath, M.G., A. Holz, D. Homann and M. Oldstone. Role of viruses in type I diabetes Seminars in Immunology, 10:87-100, 1998.

von Herrath, M.G., D. Homann, and M.B.A. Oldstone. The Role of Viruses in endocrine diseases.
In "Immunologically mediated endocrine diseases", Luciano Martini ed., in press, 2000.

von Herrath, M.G., D. Homann, J. Gairin and M.B.A. Oldstone. Pathogenesis and treatment of virus-induced autoimmune diabetes - novel insights gained through the RIP-LCMV tg mouse model Bioch. Soc. Transactions (UK) 25:630-35, 1997.

von Herrath, M.G. and M.B.A. Oldstone. Virus-induced autoimmune disease. Curr. Opinion in Immunol., 8:878-885, 1996.

Oldstone, M.B.A. and **M.G. von Herrath**. Virus-induced autoimmune disease: Transgenic approach to mimic IDDM and other autoimmune diseases. APMIS 104:689-97, 1996.

von Herrath, M.G., C. Evans, M. Horwitz and M.B.A. Oldstone. Using transgenic mouse models to dissect the pathogenesis of virus-induced autoimmune disorders of the islets of Langerhans and the central nervous system. Immunol. Reviews, 152:111-143 , 1996.

von Herrath, M.G. and M.B.A. Oldstone. Role of viruses in the loss of tolerance to self-antigens and in

the etiology of autoimmune diseases. Trends in Microbiol., 3(11):424-429, 1995.

Oldstone, M.B.A., **M.G. von Herrath**, C.F. Evans and M.S. Horwitz. Virus-Induced autoimmune disease: Transgenic approach to mimic insulin-dependent diabetes mellitus and multiple sclerosis. Curr. Top. Microbiol. Immunol., 206:67-84, 1995.

Published co-authored articles:

Sevilla, N., D. Homann, **M.G. von Herrath**, F. Rodriguez, S. Harkins, J.L. Whitton and M.B.A. Oldstone. Virus-induced diabetes in a transgenic model: Role of cross-reacting viruses and quantitation of effector T cells needed to cause disease. J. Clin. Invest., submitted, 1999.

Oldstone, M.B.A., **M.G. von Herrath**, H. Lewicki, D. Hudrisier, J.L. Whitton and J.E. Gairin. Use of a high-affinity peptide that aborts HMC-restricted cytotoxic T lymphocyte activity against multiple viruses in vitro and virus-induced immunopathologic disease in vivo. Virology 156:246-257, 1999.

Homann, D., T. Tishon, D. Berger, W. Weigle, M.G. **von Herrath**, and M.B.A. Oldstone. Evidence for an underlying CD4 helper and Cd8 T-cell defect in B-cell deficient mice. J. Virol., 72, 9208-16, 1998

Myung-Shik Lee, **M.G. von Herrath**, S. Sawyer, M. Arnush, T. Krahl, M.B.A. Oldstone and N. Sarvetnick. TGF-β fails to inhibit allograft rejection in transgenic mice. Transplantation, 7:1-10, 1996.

Lewicki, H.A., **M.G. von Herrath**, C.F. Evans, J.L. Whitton and M.B.A. Oldstone. CTL escape viral variants. II. Biologic activity in vivo. Virology, 211:443-50, 1995.

Tishon, A., H. Lewicki, G. Rall, **M.G. von Herrath** and M.B.A. Oldstone. An essential role for type 1 interferon-γ in terminating persistent viral infection. Virology, 212:244-50, 1995.

Lee, M-S., **M.G. von Herrath**, H. Reiser, M.B.A. Oldstone and N. Sarvetnick. Sensitization to self antigens by in situ expression of interferon-γ. J. Clin. Invest. 95:486-492, 1995.

Laufer, T.M., **M.G. von Herrath**, M.J. Grusby, M.B.A. Oldstone and L.H. Glimcher. Autoimmune diabetes can be induced in transgenic MHC class II deficient mice. J. Exp. Med. 178:589-596, 1993.

Published work from Ph.D. thesis:

von Herrath, M.G., G. Hasenfuss, C. Holubarsch, H. Hoffmann and H. Just. Repeated determination of left ventricles wall-thickness from mass and volume during one cardial cycle for the calculation of left ventricle wall stress parameters. Clinical Kardiologie, 13:218-220, 1990.

von Herrath, M.G. and H. Holzer. Sensitivity of fructose-1, 6-bisphosphatase from yeast, liver and skeletal muscle to F-2, 6-P2 and AMP. Zeitschrift für Lebensmittelchemie, published 1988.

Nakatani, Y., C. Banner, M. **von Herrath**, E. Schneider, H. Smith and E. Freese. Comparison of human brain and liver glutamate dehydrogenase cDNAs. Biochem. Biophys. Res. Comm. 149:405-410, 1987.

Holubarsch, Ch., G. Hasenfuss, I. Chen, M.G. **von Herrath**, M. Korner, T. Bonzel and H. Just. Myocardial performance and efficiency as assessed by energetic parameters derived from pressure-volume relations and wall-thickness in human ventricles. Zeitschrift für Kardiologie 75:100-116, 1986.

Holubarsch, Ch., G. Hasenfuss, M.G. **von Herrath**, F. Krehl, T. Bonzel and H. Just. Acute effects of nitrates on left ventricular performance and energetics. Zeitschrift für Kardiologie, 75:35-49, 1986.

Pohlig, G., W. Schafer, M.G. **von Herrath** and H. Holzer. Modification of yeast fructose-1, 6-bisphosphotase. Curr. Top. Cell. Reg., 27:317-329, 1985.

von Herrath, M.G. and H. Holzer. Oxidative inactivation of yeast fructose-1, 6-bisphosphatase. Intracell. Protein Catabolism 180:329-338, 1985.

Floyd Bloom, Professor
Chairman, Department of Neuropharmacology
Ph: 49724 Fax: 48851

Michael B.A. Oldstone, Professor
Head, Division of Virology
Department of Neuropharmacology
Ph: 48054   Fax: 49981

Lindsay Whitton, Professor
Division of Virology
Department of Neuropharmacology
Ph: 47090   Fax: 47380

Argyrios Theofilopoulos, Professor
Department of Immunology
Ph: 48138   Fax: 48361

Roger Beachy, Professor
Head, Division of Plant Biology
Ph: 42550   Fax: 42994

Speakers Invited to TSRI:

Dr. Rolf Zinkernagel
Dr. Polly Matzinger
Dr. Trevor Owens
Dr. Kevin Lafferty
Dr. C.J. Peters
Dr. Lucienne Chatenoud
Dr. Carl Djerassi
Dr. Jonathan Stoye
Dr. Marc Jenkins
Dr. Steve Miller
Dr. Burce Walker

5/1996 - Faculty Seminar, Max Planck Institute for Immunobiology, Freiburg, FRG
            Host: Professor H. Peter
5/1996 - Group Lecture, Department of Pathology, University of Cambridge, UK
            Host: Prof. Anne Cooke
12/1996 - Group Seminar, Department of Clinical Immunology, University of Freiburg,
            Host: Prof. H. Peter
6/1999 - Immunology Seminar Series, Stanford University, Host: Garrison Fathman
10/1999- Southwestrn Medical Center, Grand Rounds, Department of Dermatology, Dallas, TX, USA

37. A method for treating an autoimmune disease in a human or rodent host by suppressing an autoimmune response associated with said disease, the method comprising administering by nose or mouth to said host an effective amount for suppressing said response of a composition comprising a bystander antigen, wherein said bystander antigen is not an antigen to which T cells which mediate the disease are sensitized and wherein said bystander antigen is not an insulin antigen.

38. The method of claim 37 wherein said bystander antigen is specific to an organ or tissue afflicted by immune attack during said disease.

Cancel claim 39 without prejudice or disclaimer.

42. The method of claim 37 wherein said bystander is administered to said host in aerosol form.

43. The method of claim 37 wherein said bystander antigen is administered in a dry powder form.

44. The method of claim 37 wherein said bystander antigen is administered as a saline solution.

Cancel claim 45 without prejudice or disclaimer.

46. The method of claim 38 wherein said disease is Type I diabetes and said bystander antigen is glucagon, administered orally.

Cancel claim 47 without prejudice or disclaimer.

48. A pharmaceutical dosage form for treating an autoimmune disease in a human or rodent, the form consisting essentially of:
an effective amount for treating said disease of a bystander antigen; and a pharmaceutically acceptable carrier or diluent; wherein said bystander antigen is not insulin nor an antigen to which T cells that mediate said disease are sensitized, and wherein said dosage form is contained in an inhaler or nebulizer.

49. The pharmaceutical dosage form of claim 48 wherein said bystander antigen is specific to an organ or tissue afflicted by immune attack during said disease.

52. The pharmaceutical dosage form of claim 49 wherein said dosage form is an aerosol form.

53. The pharmaceutical dosage form of claim 49 wherein said dosage form is a saline solution.

54. The pharmaceutical dosage form of claim 49 wherein said dosage form is a dry powder.

Cancel claim 55 without prejudice or disclaimer.

56. The pharmaceutical dosage form of claim 48 wherein said disease is selected from the group consisting of Type I diabetes and animal models therefor and said bystander antigen is glucagon.

57. A pharmaceutical dosage form for nasal administration for treating Type I diabetes in a human comprising an effective amount for treating said type I diabetes of glutamic acid decarboxylase and a pharmaceutically acceptable carrier or diluent in an inhaler or nebulizer.

Cancel claims 59, 61-62 and 64-65 without prejudice or disclaimer.

M:\1010\16959\LRD0168.WPD

Suppressor T cells generated by oral tolerization to myelin basic protein suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor β after antigen-specific triggering

(tolerance/suppressor T cells/experimental autoimmune encephalomyelitis)

Ariel Miller*, Ofer Lider*, Anita B. Roberts†, Michael B. Sporn†, and Howard L. Weiner*

*Center for Neurologic Diseases, Division of Neurology, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; and †Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892

ABSTRACT   Oral administration of myelin basic protein (MBP) is an effective way of suppressing experimental autoimmune encephalomyelitis (EAE). We have previously shown that such suppression is mediated by CD8⁺ T cells, which adoptively transfer protection and suppress immune responses *in vitro*. In the present study we have found that modulator cells from animals orally tolerized to MBP produce a suppressor factor upon stimulation with MBP *in vitro* that is specifically inhibited by anti-transforming growth factor β (TGF-β) neutralizing antibodies. No effect was observed with antibodies to γ interferon, tumor necrosis factor α/β, or indomethacin. In addition, the active form of the type 1 isoform of TGF-β1 (TGF-β_1) can be directly demonstrated in the supernatants of cells from animals orally tolerized to MBP or ovalbumin after antigen stimulation *in vitro*. Antiserum specific for TGF-β_1 administered *in vivo* abrogated the protective effect of oral tolerization to MBP in EAE. Furthermore, injection of anti-TGF-β_1 serum to nontolerized EAE animals resulted in an increase in severity and duration of disease. These results suggest that immunomodulation of EAE induced by oral tolerization to MBP and natural recovery mechanisms use a common immunoregulatory pathway that is dependent on TGF-β_1. Implications of such an association are of therapeutic relevance to human autoimmune diseases and may help to explain one of the mechanisms involved in the mediation of active suppression by T cells.

Immunological tolerance is the acquisition of unresponsiveness to self antigens and as such is essential for the preservation of the integrity of the host. A variety of mechanisms underly self-tolerance, including clonal deletion, clonal anergy, and active suppression (1), and its breakdown results in autoimmune diseases. The role and mechanism of action of active suppression in regulating the immune response are not well understood. One of the classic methods of inducing tolerance is via the oral administration of antigens, first described by Wells in 1911 (2) and subsequently by Chase in 1946 (3). We and others have been studying oral tolerance as a mechanism to suppress autoimmune processes in a number of experimental models (4–11, 49) and have found that oral administration of myelin basic protein (MBP) suppresses experimental autoimmune encephalomyelitis (EAE), a central nervous system autoimmune disease mediated by CD4⁺ MBP-reactive cells (4–8). We have also found that this effect is mediated by active suppression. Specifically, CD8⁺ T cells from animals orally tolerized to MBP suppress *in vitro*

proliferative responses and adoptively transfer disease protection (5).

In the present investigation, we have found that T cells generated by oral tolerance mediate suppression both *in vitro* and *in vivo* via the release of the cytokine transforming growth factor-β (TGF-β). Our findings not only have relevance to orally induced tolerance but may help to explain one of the mechanisms by which active suppression mediated by T cells occurs.

MATERIALS AND METHODS

Animals. Female Lewis rats 6–8 weeks of age were obtained from Harlan–Sprague–Dawley. Animals were housed in Harvard Medical School animal care facilities and maintained on standard laboratory chow and water ad libitum.

Antigens. Guinea pig MBP was purified from brain tissue by the modified method of Deibler *et al.* (12). Protein content and purity were checked by gel electrophoresis and amino acid analysis.

Reagents. Commercial reagents used were as follows: mouse anti-rat γ interferon (INF-γ) neutralizing monoclonal antibody (mAb) (Amgen Biologicals); monoclonal hamster anti-murine TNF-α/β antibody (Genzyme); polyclonal rabbit neutralizing antibody against types 1 and 2 isoforms of TGF (antiTGF-β_{1+2}) (R & D Systems, Minneapolis), and indomethacin (Sigma). Turkey antiserum specific for TGF-β_1 was prepared as described (13).

Induction of Oral Tolerance. Rats were fed 1 mg of MBP dissolved in 1 ml of phosphate-buffered saline (PBS) or PBS alone by gastric intubation with a 18-gauge stainless steel animal feeding needle (Thomas Scientific). Animals were fed five times at intervals of 2–3 days with the last feeding 2 days before immunization.

***In Vitro* Suppression of Proliferative Responses by Supernatants.** Spleen cells were removed 7–14 days after the last feeding, and a single-cell suspension was prepared by pressing the spleens through a stainless steel mesh. Oral tolerance to MBP in the Lewis rat persists for ≈2 months after the last feeding (4). Thus, the 7- to 14-day period after feeding represents a relatively narrow window of time after oral tolerance is induced for performing the experiments. For preparation of supernatants, spleen cells at a concentration of 5 × 10⁶ cells per ml were stimulated *in vitro* with MBP (50 μg/ml) in 10 ml of proliferation medium as described (20). Supernatants were harvested at 24 hr, and 100 μl was added

---

Abbreviations: TGF, transforming growth factor; TGF-β_1 and -β_2, types 1 and 2 isoforms of TGF; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis; DTH, delayed-type hypersensitivity; INF-γ, γ interferon; mAb, monoclonal antibody; CFA, complete Freund's adjuvant.

to 2.5 × 10⁴ MBP-specific T cells that had been raised and maintained as described (14) and cultured with 5 × 10⁵ irradiated (2500 rad; 1 rad = 0.01 Gy) thymocytes in 100 μl of proliferation media. MBP (50 μg/ml) was added to the culture in a volume of 20 μl. Experiments were performed in triplicate in round-bottom 96-well plates (Costar). Cells were cultured for 72 hr at 37°C and harvested as described (20).

Purification of T-Cell Subsets. Depletion of lymphocyte subsets was performed by negative selection using magnetic beads according to a modified method of Cruikshank *et al.* (15). Spleen cells were incubated with a 1:10 dilution of mouse anti-rat CD8, CD4, or B-cell mAbs (clones OX/8, W3/25, or OX/33 respectively, Serotec Bioproducts) for 30 min on ice, washed twice, and then added to prewashed magnetic particles, with an average diameter of 4.5 μm (M-450) with goat anti-mouse IgG covalently attached (Dynal, Fort Lee, NJ). The cell–mAb–bead complexes were separated from unlabeled cells in a strong magnetic field with a magnetic-particle concentrator (Dynal MPC-1) for 2 min. The supernatant was removed, and the procedure was repeated twice to obtain the nonadherent fraction. The cells in the T cell- and B cell-depleted populations were >95% CD4⁺ CD8⁻, CD4⁻ CD8⁺, or CD4⁺ CD8⁺ OX/33⁻ (B cell-depleted) as demonstrated by indirect flow cytometry. Whole spleen populations (5 × 10⁶ cells) from MBP-fed or control animals were cultured in the presence of MBP (50 μg/ml) in 1 ml of serum-free proliferation media. Depleted populations were cultured at a concentration of 2.5 × 10⁶ cells per ml. Supernatants were collected at 24 hr, and 100 μl was added to responder cells as described above.

Treatment of Supernatants with Anti-Cytokine Antibodies. Spleen cells (5 × 10⁶ cells per ml in proliferation media) from MBP-fed and control animals were incubated in the presence of MBP (50 μg/ml) and neutralizing antibodies against INF-γ, TGF-β, TNF-α/β, or with indomethacin for 72 hr. Antibodies were tested in a range of concentrations (1:250, 1:500, 1:1000), and indomethacin was tested at concentrations of 0.5–1 μg/ml. At 24 hr, supernatants were collected, and free antibody or antibody–cytokine complexes were removed by using magnetizable polymer beads (Dynabeads; Dynal). Beads coupled with anti-immunoglobulin antibodies were incubated at a concentration of 4 × 10⁷ beads per ml for 30 min (done twice for each sample) and were removed by a modified method of Liabakk *et al.* (16) using a Dynal Magnetic Particle Concentrator (MPC-1).

Measurement of TGF-β Activity in Serum-Free Culture Supernatants. Serum-free culture supernatants were collected as described (17, 18). Briefly, modulator cells were first cultured for 8 hr with MBP (50 μg/ml) in proliferation medium. Thereafter, cells were washed three times, resuspended in serum-free medium for the remainder of the 72-hr culture, collected, and then frozen until assayed. Determination of TGF-β content and isoform type in supernatants was performed by using a mink lung epithelial cell line (CCL-64; American Type Culture Collection) as described by Danielpour *et al.* (13) and was confirmed by a sandwich ELISA assay as described (19). The percent active TGF-β was assayed without prior acid activation of the samples.

Immunization of Animals. Lewis rats were immunized by injection in the left foot pad with 25 μg of MBP in 50 μl of PBS emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 4 mg of mycobacterium tuberculosis (Difco) per ml.

In Vivo **Administration of Anti-TGF-β Antiserum and Control Sera.** Turkey antiserum specific for TGF-β₁ was used for *in vivo* experiments and had previously been prepared and characterized (13). Serum was heat-inactivated at 56°C for 30 min before injection. Animals (five per group) were injected i.p. with anti-TGF-β antiserum or control turkey serum at various concentrations (12.5, 25, or 50 μl diluted in PBS to a

final volume of 100 μl), five times at days −2, 0, +2, +4, and +6 in relationship to MBP/CFA immunization. One microliter of the antiserum blocked the binding activity of ¹²⁵I-labeled TGF-β₁ at 4 ng/ml to A549 cells (13). *In vivo* treatment was given both to orally tolerized animals and to animals immunized for EAE without oral tolerization.

Clinical Evaluation. Animals were evaluated in a blinded fashion every day for evidence of EAE. Clinical severity of EAE was scored as follows: 0, no disease; 1, limp tail; 2, hind limb paralysis; 3, hind limb paraplegia and incontinence; 4, tetraplegia; and 5, death.

Delayed-Type Hypersensitivity (DTH) Testing. DTH was tested by injecting 25 μg of MBP in PBS subcutaneously in the ear. Thickness was measured by a blinded observer before and 48 hr after challenge with micrometer calipers (Mitutoyo, Utsunomia, Japan).

Statistical Analysis. Clinical scales were analyzed with a two-tailed Wilcoxon rank sum test for score samples. x^2 analysis was used in comparing the incidence of disease between groups, and comparison of means was performed by using the Student t test.

RESULTS

In Vitro **Suppression Is Mediated by Culture Supernatants from CD8⁺ T Cells from MBP-Fed Animals.** In previous studies we have shown that modulator CD8⁺ splenic T cells from MBP-fed animals suppress *in vitro* proliferation of an MBP-specific T-cell line (5). Furthermore, additional studies have demonstrated that such suppression occurs when modulator and responder cells are separated by a semipermeable membrane, suggesting the presence of a soluble factor as a mediator of the suppression (20). The generation of the soluble suppressor factor requires triggering of cells from orally tolerized animals with the oral tolerogen. Experiments were thus performed to determine whether supernatants collected from splenocytes depleted of T-cell subsets or B cells from rats orally tolerized to MBP and stimulated *in vitro*
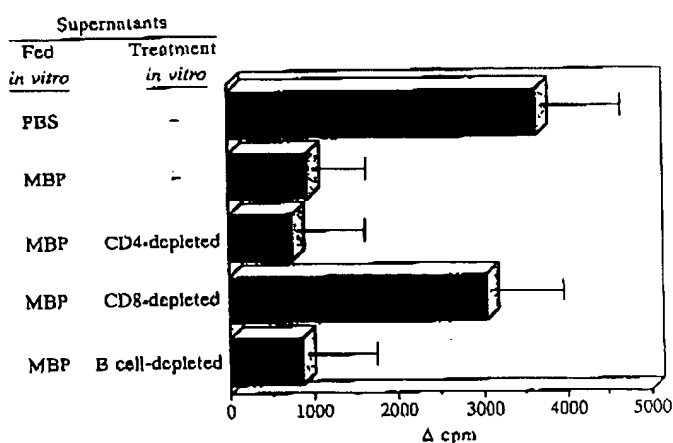
FIG. 1.   *In vitro* suppression of proliferative response mediated by supernatants of lymphocyte subsets from orally tolerized animals. Whole spleen cells or depleted cell populations were stimulated *in vitro* with MBP (50 μg/ml). Spleen cells were depleted of B lymphocytes or CD4⁺ or CD8⁺ T lymphocytes by magnetized beads. One-hundred microliters of 24-hr culture supernatants of these cells was added to 2.5 × 10⁴ MBP-specific T cells, cultured with 5 × 10⁵ irradiated (2500 rad) thymocytes in proliferation medium containing MBP (50 μg/ml). Experiments were performed in triplicate in round-bottom 96-well plates. The proliferative response of the MBP-treated line in the presence of supernatants from MBP-stimulated spleen cells of nontolerized animals was 3654 ± 1651. Background counts of the MBP treated line in the absence of MBP were between 200 and 300 cpm.

Immunology: Miller *et al.*

with MBP could suppress an MBP line. A reduction in the proliferation of the MBP line occurred with the addition of supernatants from B cell-depleted or CD4-depleted spleno-cytes from animals fed MBP and stimulated *in vitro* with MBP (Fig. 1). No suppression occurred with supernatants from cells of PBS-fed animals or CD8-depleted splenocytes from MBP-fed animals.

Inhibition of *in Vitro* Suppression by Anti-TGF-β Antibodies. To determine whether a known cytokine was responsible for mediating the suppression, neutralizing antibodies to cytokines postulated to have suppressor activity were added to the supernatants in an attempt to abrogate the suppression. Rabbit anti-TGF-β antibody abrogated the suppression mediated by the supernatants in a dose-dependent fashion (Fig. 2). No effect on suppression was seen with neutralizing antibodies to INF-γ or TNF-α/β or when indomethacin, a prostaglandin blocker, was added. No suppression occurred when anti-TGF-β antibodies were added directly to the MBP-specific responder T-cell line (data not shown).

Demonstration of TGF-β in Culture Supernatants. To directly demonstrate the presence of TGF-β in supernatants of spleen cells from animals fed MBP and stimulated *in vitro* with MBP, we collected supernatants under serum-free conditions and assayed directly for TGF-β. TGF-β was secreted by spleen cells from MBP-fed animals stimulated *in vitro* in the presence but not in the absence of MBP (Fig. 3). Furthermore, TGF-β was also secreted when splenocytes from ovalbumin-fed animals are stimulated *in vitro* with ovalbumin. By using a specific sandwich ELISA assay with blocking antibodies specific for either TGF-β₁ or TGF-β₂ (13), it was further demonstrated that the TGF-β was of the TGF-β₁ isotype. In addition, the TGF-β secreted was in the active rather than latent form. The amount of TGF-β in the group fed and stimulated *in vitro* with MBP was 6.8 ± 1.7 ng/ml, with 68 ± 9% in the active form. In the ovalbumin-fed group, the amount of TGF-β was 6.1 ± 1.0 ng/ml, with 65 ± 9% in the active form. No active TGF-β was observed in supernatants from spleen cells of animals fed MBP and stimulated with concanavalin A, although small quantities (2.1 ± 0.45 ng/ml) of latent TGF-β were observed.

FIG. 3. TGF-β activity in serum-free culture supernatants. Spleen cells from MBP- or ovalbumin (OVA)-fed animals were incubated in the presence or absence of the homologous antigen (50 μg/ml). TGF-β content in the serum-free supernatants was assayed by using a CCL-64 assay as described.

Abrogation of Oral Tolerance by *in Vivo* Administration of Anti-TGF-β Antiserum. To determine whether TGF-β₁ also played a role in suppression of EAE by oral tolerization to MBP, we administered turkey anti-TGF-β₁ antiserum *in vivo*. Paralytic EAE developed in control animals with a maximal disease severity between 3.2 and 3.5 on day 13 regardless of whether animals were injected with PBS or control turkey serum (Fig. 4A). Oral tolerization with MBP markedly reduced the severity and duration of EAE (Fig. 4C) in animals injected with PBS or control turkey serum. Maximal disease severity in animals treated five times with 50 μl of control serum was 3.2 ± 0.2 and in orally tolerized animals treated five times with 50 μl of control serum was 1.0 ± 0.2 (P < 0.05). *In vivo* treatment with anti-TGF-β₁ antiserum abro-

FIG. 4. The effect of anti-TGF-β and control sera on EAE. Animals received i.p. injections of turkey anti-TGF-β₁ antiserum or control preimmune turkey serum at various concentrations on days −2, 0, +2, +4, and +6 relative to the day of MBP/CFA challenge. Treatment was given both to orally tolerized animals (C and D) and to animals undergoing EAE without oral tolerance (A and B) (five animals per group). Maximal disease severity in orally tolerized animals treated five times with 50 μl of anti-TGF-β₁ antiserum was 3.7 ± 0.2 vs. 1.0 ± 0.2 in animals receiving control serum (P < 0.05) (D vs. C) and 4.5 ± 0.2 vs. 3.2 ± 0.2 in nontolerized animals (P < 0.05) (B vs. A). Treatments: □, PBS; ◇, control serum (12.5); ○, control serum (50); ◆, anti-TGF-β antiserum (12.5); ▲, anti-TGF-β antiserum (25); ●, anti-TGF-β antiserum (50).

FIG. 2. Inhibition of *in vitro* suppression by anti-TGF-β antibody. Spleen cells from MBP-fed and control animals were incubated in the presence of MBP (50 μg/ml) and neutralizing antibodies against INF-γ, TGF-β, TNF-α/β, or with indomethacin for 72 hr. Free antibody or antibody–cytokine complexes from 24-hr supernatants of these cells were removed by using magnetizable polymer beads as described, and the suppressive effects of the treated supernatants were tested on an MBP-specific T-cell line. The proliferative response of the MBP line in the presence of supernatants from MBP-stimulated spleen cells of nontolerized animals was 18,995 ± 2395. Background counts of the MBP line in the absence of MBP were between 1000 and 2000 cpm.

gated protection induced by oral administration of MBP in a dose-dependent fashion; maximal disease severity in orally tolerized animals treated five times with 50 μl of anti-TGF-β_1 antiserum was 3.7 ± 0.2 vs. 1.0 ± 0.2 ($P < 0.05$; Fig. 4 *D* vs. *C*). Of note is that there was a dose-dependent enhancement of disease in animals treated with anti-TGF-β_1 antiserum that were not orally tolerized to MBP (Fig. 4*B*). Disease onset was earlier, recovery was delayed, and disease severity was greater (4.5 ± 0.2 vs. 3.2 ± 0.2, Fig. 4 *B* vs. *A*; $P < 0.05$).

DTH Responses Associated with *In Vivo* Anti-TGF-β Treatment. We have shown previously that DTH responses correlate with the clinical course of EAE and serve as a measure of *in vivo* cellular immunity to MBP (6, 7). In this study prominent DTH responses developed in animals undergoing EAE and DTH was suppressed by oral administration of MBP (Fig. 5). The suppressed DTH responses were abrogated by *in vivo* anti-TGF-β_1 treatment in a dose-dependent fashion (1.5 ± 0.5 vs. 0.5 ± 0.3; $P < 0.001$, in animals injected five times with 50 μl o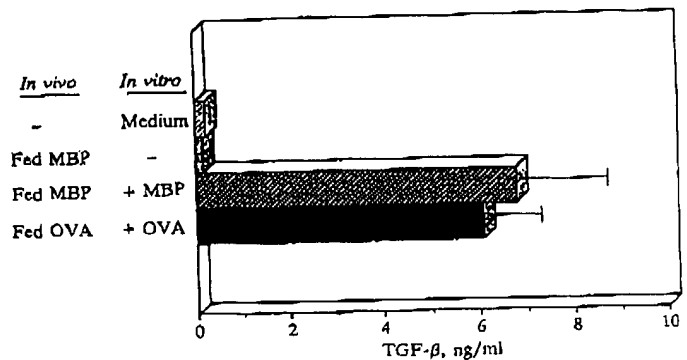f anti-TGF-β vs. control serum). Furthermore, after the same *in vivo* treatment, there was enhancement of DTH responses to MBP in animals recovering from EAE that were not orally tolerized (2.1 ± 0.3 vs. 1.45 ± 0.3; $P < 0.01$, in animals injected five times with 50 μl of anti-TGF-β vs. control serum).

DISCUSSION

In the present study we found that T cells that mediate suppression of EAE after oral tolerization to MBP do so both *in vitro* and *in vivo* via the release of active TGF-β_1. *In vitro*, the release of TGF-β is dependent on antigen-specific triggering by the oral tolerogen whether it is MBP or ovalbumin. Among the mechanisms described for the maintenance of self-tolerance, active suppression mediated by T cells is probably the least well understood (21–23). Because oral tolerization represents a physiologic pathway by which the immune system is stimulated to generate suppression and because investigators have described active suppression mediated by T cells after oral tolerization (24, 25), an understanding of the mechanism by which suppressor cells act after stimulation of the gut-associated lymphoid tissue may provide insight into the mechanism of active suppression.

A number of cytokines exert suppressive activity on different aspects of the immune response such as cell growth, differentiation, and effector functions as well as the release of

other cytokines. These include interferons, prostaglandins, tumor necrosis factor, and interleukin 10 (26). Our results show that CD8$^+$ suppressor T cells generated by oral tolerization, which are triggered in an antigen-specific fashion, act both *in vitro* and *in vivo* by the release of active TGF-β_1. In this regard they are analogous to the human CD8$^+$ suppressor cells associated with lepromatous leprosy, which act via an as-yet-unidentified antigen nonspecific factor after being triggered by a specific antigen (27). We recently have found that peripheral blood lymphocytes from humans orally tolerized to keyhole limpet hemocyaninin secrete a TGF-β-related suppressor factor after antigen-specific triggering (51). Because suppression generated by oral tolerance to autoantigens is antigen- and disease-specific, the secretion and action of TGF-β must occur in the local microenvironment of lymphoid tissue where the immune response is generated, along migratory pathways of the effector cells, and/or at the inflamed site in the target organ where the autoantigen is present. We recently have found specific elevation of TGF-β in the brains of EAE animals orally tolerized to MBP as compared to nontolerized animals (S. J. Khoury, W. W. Hancock, and H.L.W., unpublished observations). The ability of T cells to secrete TGF-β_1 in an active form may be important in this regard since active TGF-β has a short half-life and a small volume distribution, but latent TGF-β has an extended half-life and a larger volume of distribution (28).

We have also observed that injection of anti-TGF-β_1 serum to nontolerized animals immunized with MBP/CFA to induce EAE resulted in an increase in severity and duration of EAE. Other investigators have reported the presence of postrecovery CD4$^+$ suppressor cells in the Lewis rat EAE model that can adoptively transfer protection and suppress *in vitro* proliferative responses of an MBP line (29). *In vitro*, these cells have been shown to release TGF-β and to suppress interleukin-2 and IFN-γ production by encephalitogenic cells (29), although an *in vivo* effect of TGF-β by such cells has not been shown. Our *in vivo* results show a role for TGF-β in recovery from EAE. Thus, natural recovery in EAE and the induction of oral tolerance to MBP, though two distinct physiological processes, appear to involve a common immunoregulatory pathway in which TGF-β serves as an effector cytokine. The role of TGF-β-secreting CD8$^+$ T cells as opposed to CD4$^+$ T cells in natural recovery remains to be defined. TGF-β itself, when administered systematically in doses of 1–5 μg per treatment, has been shown to suppress
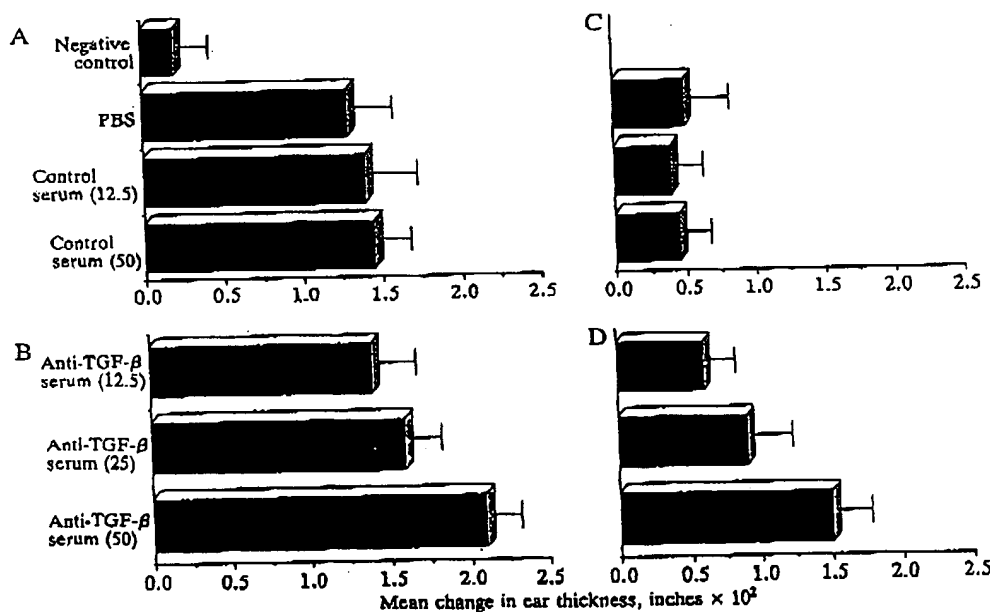
FIG. 5. DTH responses associated with *in vivo* anti-TGF-β treatment. DTH was tested in the groups described in Fig. 4 by injecting 25 μg of MBP in PBS subcutaneously in the ear. Thickness in inches (1 in. = 2.54 cm) was measured before and 48 hr after challenge. Change in ear thickness before and after challenge was recorded for each animal, and the results are expressed as the mean for each experimental group ± SEM. [$P < 0.001$, animals treated five times with 50 μl of anti-TGF-β_1 antiserum vs. control serum (D vs. C); $P < 0.01$, animals treated five times with 50 μl of anti-TGF-β_1 antiserum vs. control serum (B vs. A).]

animal models of autoimmunity both in the rat and mouse, including EAE (30, 31) and autoimmune arthritis (32, 33).

The mechanism by which TGF-β suppresses immune responses *in vitro* and down-regulates EAE and other autoimmune diseases *in vivo* is unknown. Recent studies have demonstrated multiple, sometimes contradictory, immunomodulatory effects of the TGF-β isoforms on various target cells and tissues (34, 35). Although initially identified as a growth factor, the immunoregulatory properties of TGF-β include inhibition of proliferation of B and T cells, (16, 36) affecting CD4$^+$ cells more than CD8$^+$ cells (37) in both rodent and human cells. TGF-β antagonizes inflammatory effector cytokines such as TNF-α and INF-γ (38, 39), blocks CTL activity (40, 50), and inhibits induction of receptors of interleukins 1 and 2 (41), rendering cells unresponsive to these cytokines. TGF-β inhibits *in vivo* T-cell and neutrophil adhesion to endothelial cells, which limits the migration and recruitment of inflammatory cells into the target organ (42, 43), downregulates class II expression on macrophages and astrocytes, and inhibits macrophage activation (40, 44).

TGF-β has been demonstrated to be secreted by a variety of cells including macrophages, natural killer cells, LAK cells, B cells, and both CD4$^+$ and CD8$^+$ T cells (45–47). The characteristics of the CD8$^+$ suppressor cells generated after oral tolerization are yet to be defined. Concanavalin A stimulation of spleen cells from animals tolerized with MBP, as opposed to MBP stimulation, did not lead to significant suppressor effects nor production of active TGF-β. This may be due to induction of proliferation rather than production of TGF-β after concanavalin A stimulation (48).

The results presented herein provide evidence for an immunoregulatory role played by endogenous TGF-β₁ in the spontaneously occurring recovery from EAE and in the suppression of EAE induced by oral tolerization to MBP. In view of its evolutionary high conserved features, it is likely that the immunosuppressive effects of TGF-β in experimental animals are similar to its effects in humans. Thus, our findings may point to the therapeutic potential of oral tolerization to MBP or other autoantigens as a source of endogenous TGF-β for the control of autoimmune diseases.

1. Schwartz, R. H. (1989) *Cell* **57**, 1073–1081.
2. Wells, H. G. (1911) *J. Infect. Dis.* **9**, 147–171.
3. Chase, M. W. (1946) *Proc. Soc. Exp. Biol. Med.* **61**, 257–259.
4. Higgins, P. J. & Weiner, H. L. (1988) *J. Immunol.* **140**, 440–445.
5. Lider, O., Santos, L. M. B., Lee, C. S. Y., Higgins, P. J. & Weiner, H. L. (1989) *J. Immunol.* **142**, 748–752.
6. Brod, S. A., Al-Sabbagh, A., Sobel, R. A., Hafler, D. A. & Weiner, H. L. (1991) *Ann. Neurol.* **29**, 615–622.
7. Khoury, S. J., Lider, O., Al-Sabbagh, A. & Weiner, H. L. (1990) *Cell. Immunol.* **131**, 302–310.
8. Bitar, D. & Whitacre, C. C. (1988) *Cell. Immunol.* **112**, 364–370.
9. Nussenblatt, R. B., Caspi, R. R., Mahdi, R., Chan, C.-C., Roberge, F., Lider, O. & Weiner, H. L. (1989) *J. Immunol.* **144**, 1689–1695.
10. Zang, Z. J., Lee, C. S. Y., Lider, O. & Weiner, H. L. (1990) *J. Immunol.* **145**, 2489–2493.
11. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. & Thorbecke, F. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7443–7446.
12. Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2**, 139–165.
13. Danielpour, D., Dart, L. L., Flanders, K. C., Roberts, A. B. & Sporn, M. B. (1989) *J. Cell. Physiol.* **138**, 79–86.
14. Ben-Nun, A., Wekerle, H. & Cohen, I. R. (1981) *Eur. J. Immunol.* **11**, 195–199.
15. Cruikshank, W. W., Berman, J. S., Theodor, A. C., Bernardo, J. & Center, D. M. (1987) *J. Immunol.* **138**, 3817–3823.
16. Liabakk, N.-B., Nustad, K. & Esekevik, T. (1989) *Scand. J. Immunol.* **30**, 641.
17. Kehrl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. S. (1986) *J. Exp. Med.* **163**, 1037–1050.
18. Wahl, S. M., Allen, J. B., Wong, H. L., Dougherty, S. F. & Ellingsworth, L. R. (1990) *J. Immunol.* **145**, 2514–2519.
19. Danielpour, D., Kim, K.-Y., Dart, L. L., Watanabe, S., Roberts, A. B. & Sporn, M. B. (1989) *Growth Factors* **2**, 61–71.
20. Miller, A., Lider, O. & Weiner, H. L. (1991) *J. Exp. Med.* **174**, 791–798.
21. Gershon, R. K. & Kondo, K. (1971) *Immunology* **21**, 903–914.
22. Dorf, M. E. & Benacerraf, B. (1984) *Annu. Rev. Immunol.* **2**, 127–158.
23. Tada, T., Asano, Y. & Sano, K. (1989) *Res. Immunol.* **140**, 291–294.
24. Miller, S. D. & Hanson, D. G. (1979) *J. Immunol.* **123**, 2344–2350.
25. Gautam, S. & Battisto, J. R. (1985) *J. Immunol.* **135**, 2975–2983.
26. Cohen, S., ed. (1990) *Lymphokines and the Immune Response* (CRC, Boca Raton, FL).
27. Salgame, P., Modlin, R. L. & Bloom, B. R. (1989) *Int. Immunol.* **1**, 121–129.
28. Wakefield, L. M., Winokur, T. S., Hollands, R. S., Christopher-son, K., Levinson, A. D. & Sporn, M. B. (1990) *J. Clin. Invest.* **86**, 1976–1984.
29. Karpus, W. J. & Swanborg, R. H. (1991) *J. Immunol.* **146**, 1163–1168.
30. Racke, M. K., Dhib-Jalbut, S., Cannella, B., Albert, P. S., Raine, C. S. & McFarlin, D. E. (1991) *J. Immunol.* **146**, 3012–3017.
31. Johns, L. D., Flanders, K. C., Range, G. E. & Sriram, S. (1991) *J. Immunol.* **147**, 1792–1796.
32. Kuruvilla, A. P., Shah, R., Hochwald, G. M., Liggett, H. D., Palladino, M. A. & Thornbecke, G. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2918–2921.
33. Brandes, M. E., Allen, J. B., Ogawa, Y. & Wahl, S. M. (1991) *J. Clin. Invest.* **87**, 1108–1113.
34. Sporn, M. B., Roberts, A. B., Wakefield, L. M. & Assoian, R. K. (1986) *Science* **233**, 532–534.
35. Wahl, S. M., McCartney-Francis, N. & Mergenhagen, S. E. (1989) *Immunol. Today* **10**, 258–261.
36. Kehrl, J. H., Roberts, A. B., Wakefield, L. M., Jakowlew, S., Sporn, M. B. & Fauci, A. S. (1986) *J. Immunol.* **137**, 3855–3860.
37. Tada, T., Ohzeki, S., Utsumi, K., Takiuchi, H., Muramatsu, M., Li, X.-F., Shimizu, J., Fujiwara, H. & Hamaoka, T. (1991) *J. Immunol.* **146**, 1077–1082.
38. Espevik, T., Figaro, I. S., Shalaby, M. R., Lackides, G. A., Lewis, G. D., Shepard, H. M. & Palladino, M. A. (1987) *J. Exp. Med.* **166**, 571–576.
39. Schluesener, H. J. (1990) *J. Neuroimmunol.* **27**, 41–47.
40. Ranges, G. E., Figari, I. S., Espevik, T. & Palladino, M. A. (1987) *J. Exp. Med.* **166**, 991–998.
41. Dubois, C. M., Ruscetti, F. W., Palaszynski, E. W., Falk, L. A., Oppenheim, J. J. & Keller, J. R. (1990) *J. Exp. Med.* **172**, 737–744.
42. Gamble, J. R. & Vadas, M. A. (1991) *J. Immunol.* **146**, 1149–1154.
43. Gamble, J. R. & Vadas, M. A. (1988) *Science* **242**, 97–99.
44. Ding, A., Nathan, C. F., Graycar, J., Serynck, R., Stuehr, D. J. & Srimal, S. (1991) *J. Immunol.* **145**, 940–944.
45. Assoian, R. K., Fleurdelys, B. E., Stevenson, H. C., Miller, P. J., Madtes, D. K., Raines, E. W., Ross, R. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6020–6025.
46. Kasid, A., Bell, G. I. & Director, E. P. (1988) *J. Immunol.* **141**, 690–698.
47. Kim, S.-J., Kehrl, J. H., Burton, J., Tendler, C. L., Jeang, K.-T., Danielpour, D., Thevenin, C., Kim, K. Y., Sporn, M. B. & Roberts, A. B. (1990) *J. Exp. Med.* **172**, 121–129.
48. Bristol, L. A., Ruscetti, F. W., Brody, D. T. & Durum, S. K. (1990) *J. Immunol.* **145**, 4108–4114.
49. Zhang, Z. J., Davidson, L., Eisenbarth, G. & Weiner, H. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10252–10256.
50. Fontana, A., Frei, K., Bodmer, S., Hofer, E., Schreier, M. H., Pallidino, M. A. & Zinkernagel, R. M. (1989) *J. Immunol.* **143**, 3230–3234.
51. Matsui, M., Miller, A., Hafler, D. A. & Weiner, H. L. (1991) *J. Neuroimmunol.* (Suppl. 1), 85 (abstr.).

2/4/92

CURRICULUM VITAE

NAME:	George Stephen Eisenbarth
ADDRESS:	8 Summit Road, Wellesley, MA 02181
DATE OF BIRTH:	September 17, 1947
PLACE OF BIRTH:	Brooklyn, New York

EDUCATION
1969	B.A., Columbia University, New York, NY
1974	Ph.D., Duke University, Durham, NC (Physiology-Pharmacology)
1975	M.D., Duke University School of Medicine

INTERNSHIP AND RESIDENCIES AND FELLOWSHIPS
1975	Intern, Duke University Medical Center
1976	Junior Assistant Resident in Medicine, Duke University Medical Center
1/1977-6/77	Fellow in Endocrinology, Duke University Medical Center
1977-79	Research Associate, National Heart, Lung and Blood Institute, Laboratory of Biochemical Genetics (M. Nirenberg, A. Fauci), NIH, Bethesda, MD
1977-79	Fellow, Combined Endocrinology Program Clinic, NIH

LICENSURE AND CERTIFICATION
1976	National Board of Medical Examiners, No. 126840
1978	American Board of Internal Medicine, No. 63999
1979	Diplomate, Endocrinology and Metabolism, No. 63999
1982	Massachusetts License, Registration No. 49856

ACADEMIC APPOINTMENTS
1979-82	Assistant Professor of Medicine & Physiology, Duke University
1982-	Associate Professor of Medicine, Harvard Medical School, Boston, MA

HOSPITAL APPOINTMENTS
1979-82	Assistant Professor of Medicine, Duke University
1982-	Associate Physician, Brigham and Women's Hospital, Harvard Medical School
1983-	Associate on Staff, New England Deaconess Hospital, Boston, MA

1

...G.S.Eisenbarth, M.d., Ph.D.

OTHER PROFESSIONAL POSITIONS

1982-	Senior Investigator, Elliot P. Joslin Research Laboratories, Joslin Diabetes Center, Boston, MA
1982-	Head, Immunology Section, Research Division, Joslin Diabetes Center
1987-1988	Chairman, American Diabetes Association Research Committee
1987-1991	Member, Immunologic Sciences Study Section, NIH
1990-	Chairman elect Immunology of Diabetes Workshop (IDW) Committee
1990-	Consultant Calbio, Immunologic, Xoma, Autoimmune
1990-1991	Acting Chief, Diabetes Division, Brigham and Women's Hospital, Boston, MA
1991-	Advisory Board Duke University Arthritis Center

AWARDS AND HONORS

1965	Pulitzer Scholarship, Columbia University, NY
1981-84	Career Development Awardee, Juvenile Diabetes Foundation
1984	Weitzman Memorial Award, American Endocrine Society
1986	Outstanding Scientific Achievement Award of the American Diabetes Association and the Lilly Lecture
1987	JDF Mary Jane Kugel Award
1987	Peter J. Laurie Memorial Lecture
1988	JDF Don Silver Excellence in Research Award
1989	Louise Buerki Visiting Professor, Henry Ford Hospital
1989	Fourteenth Annual Arnold Lazarow Memorial Lecture, University of Minnesota
1990	Otto Brandman Award, ADA, New Jersey Affiliate

MEMBERSHIPS, OFFICES AND COMMITTEE ASSIGNMENTS

1979	American College of Physicians
	American Federation of Clinical Research
	The Endocrine Society
1982	American Society of Clinical Investigation
	American Diabetes Association
1983-88	American Diabetes Association Research Committee
1983-85	Editorial Board Diabetes
1984-87	Juvenile Diabetes Foundation, Medical Science Review Committee
1985	Second Vice Chairman, American Diabetes Association Research Committee
1987	Chairman, American Diabetes Association, Research Committee
1985-88	Treasurer IDW (Immunology of Diabetes Workshop)

MEMBERSHIPS, OFFICES AND COMMITTEE ASSIGNMENTS (Contd.)

1987-89	American Diabetes Association Research Policy Committee
1987	Chairman, ADA Research Symposium, VIII International Conference on the Immunology of Diabetes, Woods Hole, MA
1986-	Scientific Board, Barbara Davis Childhood Diabetes Center, Denver, Colorado
1987-91	Immunologic Sciences Study Section, National Institutes of Health
1986-	Board of Governors, American Diabetes Association, Massachusetts Affiliate
1987-	Editorial Board, Journal of Autoimmunity
1986-89	Board of Trustees, Joslin Diabetes Center
1986-	Associate Director, and Director of Cores, Diabetes Endocrine Research Center, Joslin Diabetes Center
1987-	Metablo Scientific Advisory Board
1988-	Editorial Board, Journal of Clinical Immunology
1988-91	Chairman-Elect, Immunology of Diabetes Workshops Committee
1988-91	Chairman Abstract Subcommittee of the 14th International Diabetes Federation Congress, Washington DC
1989-90	National Diabetes Advisory Council, Diabetes Treatment Centers of America Foundation
1989-	Consultant, CW Group
1989-	Consultant, Xoma Corporation
1989-1990	National Institutes of Allergy and Infectious Diseases Task Force on Immunology and Allergy, chapter co-chair
1990	Chairman Elect IDW Committee
1990	CRC Advisory Committee Brigham and Womens
1991-	Associate Editor, Diabetes
1991-95	National Institute of Health Reviewers Reserve.
1991	External Advisor, Duke Specialized Center of Research in Rheumatoid Arthritis.

MAJOR RESEARCH INTERESTS

Immunoendocrinology
Immunogenetics
Islet Cell Culture

TEACHING EXPERIENCE

1979-82	Lecturer for the Physiology and Endocrinology courses to medical students at Duke University
1979-82	Attending Physician, Medical Service, Duke University
1979-82	Coordinator, Endocrine Research Conference, Duke University Medical Center
1982-	Endocrinology/Diabetes Consultant, Brigham & Women's Hospital
1983-84	Coordinator, Joslin Research Seminar
1983-	Attending Physician, Medical Service, New England Deaconess Hospital
1985-	Attending Diabetes Treatment Unit, Joslin Diabetes Center
1989-	Invited Lecturer, Pharmacology Medical Student Course, MIT
1991	Max Miller Lecture, 2nd Annual Midwest Metabolism Club Meeting, The University of Chicago Medical Center

PRINCIPAL CLINICAL AND HOSPITAL SERVICE RESPONSIBILITIES

1983-85	Human Investigations Committee, New England Deaconess Hospital
1983-85	Human Investigations Committee, Joslin Diabetes Center
1984-85	Bylaws Committee, Joslin Diabetes Center
1985-88	Operations Committee, Joslin Diabetes Center
1989-	Institutional Review Board, Brigham and Womens Hospital
1989-	Institutional Review Board, Brigham and Womens Hospital
1992-	McLaughlin Lecture, The University of Texas Medical Branch at Galveston

G.S. Eisenbarth, M.d., Ph.D.

ORIGINAL REPORTS

1. Delcher HK, Eisenbarth GS, Lebovitz HE. Fatty acid inhibition of sulfation factor stimulated 35S04 incorporation to embryonic chick cartilage. J Biol Chem 1973; 248:1901-1905.

2. Birch BM, Delcher HK, Rendall JL, Eisenbarth GS, Lebovitz HE. Evidence that endogenous cyclic AMP does not modulate serum sulfation factor action on embryonic chicken cartilage. Biochem Biophys Res Comm 1973; 52:1184-1189.

3. Eisenbarth GS, Beuttel SC, Lebovitz HE. Fatty acid inhibition of somatomedin (serum sulfation factor) stimulated protein and RNA synthesis in embryonic chicken cartilage. Biochem Biophys Acta 1973; 331:397-409.

4. Eisenbarth GS, Lebovitz HE. Prostaglandin inhibition of cartilage chrondromucoprotein synthesis: concept of intrinsic activity. Prostaglandins 1974; 7:11-20.

5. Eisenbarth GS, Beuttel SC, Lebovitz HE. Inhibition of cartilage macromolecular synthesis by prostaglandin A1. J Pharmacol Exp Ther 1974; 189:213-220.

6. Eisenbarth GS, Lebovitz HE. Isolation and characterization of a serum inhibitor of cartilage metabolism. Endocrinology 1974; 95:1600-1607.

7. Eisenbarth GS, Wellman KD, Lebovitz HE. Prostaglandin A1 inhibition of chondrosarcoma growth. Biochem Biophys Res Comm 1974; 60:1302-1308.

8. Drezner MK, Eisenbarth GS, Neelon FA, Lebovitz HE. Stimulation of cartilage amino acid uptake by growth hormone-dependent factors in serum: mediation of adenosine 3', 5'-monophosphate. Biochem Biophys Acta 1975; 381-384.

9. Eisenbarth GS, Drezner MK, Lebovitz HE. Inhibition of chondromucoprotein synthesis: an extraneuronal effect of nerve growth factor. J Pharmacol Exp Ther 1975; 192:630-634.

10. Beuttel SC, Eisenbarth GS, Lebovitz HE. Amino acid dependent and independent insulin stimulation of cartilage metabolism. Biochemistry 1977; 16:5759-5764.

11. Eisenbarth GS, Wilson P, Ward F, Lebovitz HE. HLA type and disease occurrence in familial poly-glandular failure. N Engl J Med 1978; 298:92-94.

12. Ruffolo R, Eisenbarth GS, Thompson J, Nirenberg M. Synapse turnover: a mechanism for acquiring synaptic specificity. Proc Natl Acad Sci (USA) 1978; 75:2281-2285.

....*G.S. Eisenbarth, M.d., Ph.D.*

ORIGINAL REPORTS (Contd.)

13. Eisenbarth GS, Ruffolo R, Walsh F, Nirenberg M. Lactose sensitive lectin of chick retina and spinal cord. Biochem Biophys Res Comm 1978; 83:1246-1252.

14. Eisenbarth GS, Walsh R, Nirenberg M. Monoclonal antibody to a plasma membrane antigen of neurons. Proc Natl Acad Sci (USA) 1979; 76:4913-4917.

15. Eisenbarth GS, Wilson P, Ward F, Buckley C, Lebovitz HE. The polyglandular failure syndrome: disease inheritance, HLA-type and immune function. Studies in patients and families. Ann Int Med 1979; 91:528-533.

16. Schneider M, Eisenbarth GS. Transfer plate radioassay using cell monolayers to detect anti-cell surface antibodies synthesized by lymphocyte hybridomas. J Immunol Methods 1979; 29:311-342.

17. Haynes BF, Eisenbarth GS, Fauci AS. Human lymphocyte antigens: production of a monoclonal antibody which defines functional thymus-derived lymphocyte subsets. Proc Natl Acad Sci (USA) 1979; 76:5829-5833.

18. Nirenberg M, Wilson S, Ignashida H, Thompson J, Eisenbarth GS, Walsh F, Rotter A, Kenimer J, Sabol S. Synapse plasticity. Scripta Varia 1979.

19. Eisenbarth GS, Haynes BF, Schroer JA, Fauci AS. Production of monoclonal antibodies reacting with peripheral blood mononuclear cell surface differentiated antigens. J Immunol 1980; 124:1237-1244.

20. Eisenbarth GS, Rankin B, Haynes BF, Fauci AS. Visual assay to monitor purification of cell surface antigens reacting with monoclonal antibodies. J Immunol Methods 1980; 39:387-392.

21. Haynes BF, Mann DL, Hemler ME, Schroer JA, Shelhamer JH, Eisenbarth GS, Strominger JL, Thomas CA, Mostowski HS, Fauci AS. Characterization of a monoclonal antibody that defines a immunoregulatory T cell subset for immunoglobulin synthesis in humans. Proc Natl Acad Sci (USA) 1980; 7:2914-2918.

22. Wilson PW, Buckley CE III, Eisenbarth GS. Disordered immune function in patients with polyglandular failure. J Clin Endo Metab 1981; 51:284-288.

23. Haynes BF, Bunn P, Mann D, Thomas C, Eisenbarth GS, Minna J, Fauci AS. Cell Surface differentiation antigens of the malignant T cell in Sezary syndrome and mycosis fungoids. J Clin Invest 1981; 67:523-530.

G.S.Eisenbarth,M.d.,Ph.D.

ORIGINAL REPORTS (Contd.)

24. Eisenbarth GS, Oie H, Gazdar Z, Chick WL, Schultz JA, Scearce RM. Production of monoclonal antibodies reacting with rat islet cell membrane antigens. Diabetes 1981; 30:226-230.

25. Scearce RM, Oie HK, Gazdar AF, Chick WL, Eisenbarth GS. Transfer plate radioassay using absorbed anti-insulin antibody to detect insulin secreted by islet cell cultures. J Immunol Methods 1981; 42:27-33.

26. Eisenbarth GS, Morris MA, Scearce RM. Cytotoxic antibodies to cloned rat islet cells in serum of patients with diabetes mellitus. J Clin Invest 1981; 67:403-408.

27. Verghese MW, Ward FE, Eisenbarth GS. Lymphocyte suppressor activity in patients with polyglandular failure. Human Immunol 1981; 3:173-179.

28. Haynes BF, Hemler ME, Mann DL, Eisenbarth GS, Shelhamer J, Mostowski HS, Thomas CA, Strominger JL, Fauci AS. Characterization of a monoclonal antibody (4F2) that binds to human monocytes and to a subset of activated lymphocytes. J Immunol 1981; 126:1409-1414.

29. Haynes BF, Hemler M, Cotner T, Mann DL, Eisenbarth GS, Strominger JL, Fauci AS. Characterization of a monoclonal antibody (5E9) which defines a human cell surface antigen of cell activation. J Immunol 1981; 127:347-351.

30. Jackson R, Rassi N, Crump A, Haynes BF, Eisenbarth GS. The BB diabetic rat: profound T cell lymphocytopenia. Diabetes 1981; 30:887-889.

31. Shimizu K, Eisenbarth GS, Wells S. Expression of receptors for tetanus toxin and monoclonal antibody A2B5 on "APUD" cells and "APUD" cell tumors. Surg Forum 1981; 32:419-421.

32. Eisenbarth GS, Shimizu K, Bowring MA, Wells S. Expression of receptors for tetanus toxin and monoclonal antibody A2B5 by pancreatic islet cells. Proc Natl Acad Sci (USA) 1982; 79:5066-5070.

33. Rowley RF, Eisenbarth GS. Selective binding of fluoresceinated-avidin to A cells of sectioned pancreas. Diabetes 1982; 31:107-109.

34. Haynes BF, Reisner EG, Hemler ME, Strominger JL, Eisenbarth GS. Description of a monoclonal antibody defining an HLA allotypic determinant that included specificities within the B5 cross-reacting group. Human Immunol 1982; 4:273-285.

35. Jackson RA, Morris MA, Haynes BF, Eisenbarth GS. Increased circulating Ia-antigen-bearing T cells in Type I diabetes mellitus. N Engl J Med 1982; 306:785-788.

...G.S. Eisenbarth, M.d., Ph.D.

ORIGINAL REPORTS (Contd.)

36. Shimizu K, Reintgen D, Rowley R, Coleman E, Briner W, Sieger H. Eisenbarth GS. In vivo and in vitro binding of iodinated-monoclonal antibody A2B56 to RIN insulinoma cells. Hybridoma 1982; 2(1):69-77.

37. Eisenbarth GS, Linnenbach A, Jackson RA, Scearce R, Croce C. Human hybridomas secreting anti-islet autoantibodies. Nature 1982; 300:264-267.

38. Kortz W. Reiman TH, Bollinger R, Eisenbarth GS. Identification and isolation of rat and human islet cells using monoclonal antibodies. Surg Forum 1982; 33:354-356.

39. Jackson R, Kadison P, Buse J, Rassi N, Jegosthy B, Eisenbarth GS. Lymphocyte abnormalities in the BB rat. Metabolism 1982; 32(7):83-86.

40. Crump A, Scearce R, Doberson M, Kortz WJ, Eisenbarth GS. Production and characterization of a cytotoxic monoclonal antibody reacting with rat islet cells. J Clin Invest 1982; 70:659-666.

41. Reintgen D. Shimizu K, Coleman E, Eisenbarth GS, Briner W, Seigler HF. In vivo tumor localization using radiolabeled monoclonal antibody A2B5. Surg Forum 1982; 33:431-434.

42. Haynes BF, Shimizu K, Eisenbarth GS. Identification of human and rodent thymic epithelium using tetanus toxin and monoclonal antibody A2B5. J Clin Invest 1983; 71:9-14.

43. Srikanta S, Ganda OP, Eisenbarth GS, Soeldner JS. Islet cell antibodies and beta cell function in monozygotic triplets and twins initially discordant for Type I diabetes mellitus. N Engl J Med 1983; 308:322-325.

44. Telen MJ, Eisenbarth GS, Haynes BF. Human erythrocyte antigens: regulation of expression of a novel erythrocyte surface antigen by the inhibitor Lutheran In(Lu) gene. J CLin Invest 1983; 71:1878-1886.

45. Thompson JM, Eisenbarth GS, Ruffolo Jr, RR, Nirenberg M. Synapse selection based on differences in synapse turnover. Intl J Devel Neurosci 1983; 1:25-30.

46. Srikanta S. Ganda OP. Jackson RA. Gleason RE. Kaldany A. Garovoy MR. Milford EL. Carpenter CB, Soeldner JS, Eisenbarth GS. Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. Ann Int Med 1983; 99:320-326.

47. Haynes BF, Warren RW, Buckley RH, McClure JE, Goldstein AL, Henderson FW, Hensley LL, Eisenbarth GS. Demonstration of abnormalities in expression of thymic epithelial surface antigens in severe cellular immunodeficiency diseases. J Immunol 1983; 130:1182-1188.

....*G.S. Eisenbarth, M.d., Ph.D.*

ORIGINAL REPORTS (Contd.)

48. Haynes BF, Harden EA, Olanow CW, Eisenbarth GS, Wechsler A, Hensley LL, Roses AD. Effect of thymectomy on peripheral lymphocyte subsets in myasthenia gravis: selective effect on T cells in patients with thymic atrophy. J Immunol 1983; 131:773-777.

49. Haynes BF, Harden EA, Telen MJ, Hemler ME, Strominger JL, Palker TJ, Scearce RM, Eisenbarth GS. Differentiation of human T lymphocytes. I. Acquisition of a novel human cell surface protein (p80) during normal intrathymic T cell maturation. J Immunol 1983; 131:1195-1200.

50. Reintgen DS, Shimizu K, Coleman E, Briner W, Kitzmiller J, Eisenbarth GS, Seigler HF. Immunodiagnosis of tumors in vivo using radiolabeled monoclonal antibody A2B5. J Surg Oncol 1983; 23(3):205-211.

51. Jackson RA, Haynes BF, Burch WM, Shimizu K, Bowring MA, Eisenbarth GS. Ia+ T cell in new onset Graves' disease. J Clin Endo Metab 1984; 59:187-190.

52. Powers AC, Rabizadeh A, Akeson R, Eisenbarth GS. Characterization of monoclonal 3G5 and utilization of this antibody to immobilize pancreatic islet cell gangliosides in a solid phase radioassay. Endocrinology 1984; 114:1-6.

53. Ganda OP, Srikanta S, Brink WJ, Morris MA, Gleason RE, Soeldner JS, Eisenbarth GS. Differential Sensitivity to beta cell secretagogues in "early" Type I diabetes. Diabetes 1984; 33:516-521.

54. Jackson RA, Buse JB, Rifai R, Pelletier D, Milford EL, Carpenter CB, Eisenbarth GS, Williams RM. Two Genes required for diabetes in BB rats. J Exp Med 1984; 159:1629-1631.

55. Srikanta S, Ganda OP, Gleason RE, Jackson RA, Soeldner JS, Eisenbarth GS. Pre-Type I diabetes: linear loss of beta cell response to intravenous glucose. Diabetes 1984; 33:717-720.

56. Buse JB, Ben-Nun A, Klein KA, Eisenbarth GS, Seidman JG, Jackson RA. Specific class II histocompatibility gene polymorphism in BB rats. Diabetes 1984; 33:700-703.

57. Srikanta S, Ganda OP, Jackson RA, Brink SJ, Fleischnick E, Yunis E, Alper C, Soeldner JS, Eisenbarth GS. Pre-Type I diabetes: common endocrinologic course despite immunologic and immunogenetic heterogeneity. Diabetologia 1984; 27:146-148.

58. Buse JB, Ben-Nun A, Klein KA, Eisenbarth GS, Seidman JG, Jackson RA. Class I, II and III major histocompatibility complex gene polymorphisms in BB rats. Diabetologia 1984; 22:77-79.

59. Srikanta S, Eisenbarth GS. Disappearing anti-islet antibodies? Lancet 1984: i:1176-1177.

....*G.S. Eisenbarth, M.d., Ph.D.*

ORIGINAL REPORTS (Contd.)

60. Srikanta S, Rabizadeh A, Omar MAK, Eisenbarth GS. Assay for islet cell antibodies: protein a - monoclonal antibody method. Diabetes 1985; 34:300-305.

61. Maron R, Jackson RA, Jacobs S, Eisenbarth GS, Kahn CR. Analysis of the insulin receptor by anti-receptor antibodies and flow cytometry. Proc Natl Acad Sci (USA) 1985; 81:7446-7450.

62. Eisenbarth GS, Fleischnick E, Ganda OP, Srikanta S, Jackson RA, Brink SJ, Soeldner JS, Yunis E, Alper C. Progressive autoimmune beta cell insufficiency: occurrence in the absence of high risk HLA alleles DR3, DR4. Diabetes Care 1985; 8:477-480.

63. Nayak RC, Omar MAK, Rabizadeh A, Srikanta S, Eisenbarth GS. "Cytoplasmic" islet cell antibodies: evidence that the target antigen is a sialoglycoconjugate. Diabetes 1985; 34:617-619.

64. Buse JB, Rifai-Haddad R, Lees S, Taniguchi H, Chaplin D, Milford E, Seidman J, Eisenbarth GS, Jackson RA. Major histocompatibility complex restriction fragment length polymorphisms define three diabetogenic haplotypes in BB and BBN rats. J Exp Med 1985; 162:444-458.

65. Srikanta S, Ganda OP, Soeldner JS, Eisenbarth GS. First degree relatives of patients with Type I diabetes mellitus: islet cell antibodies and abnormal insulin secretion. N Engl J Med 1985; 313:461-464.

66. Eisenbarth GS, Srikanta S, Jackson RA, Rabinowe SL, Dolinar R, Haynes BF, Morris MA. ATGAM and prednisone immunotherapy of recent onset Type I diabetes mellitus. Diabetes Res 1985; 2:271-276.

67. Soeldner JS, Tuttleman M, Srikanta S, Ganda OP, Eisenbarth GS. Insulin dependent diabetes mellitus and initiation of autoimmunity: islet cell autoantibodies, insulin autoantibodies and beta cell failure. N Engl J Med 1985; 313(14):893-894.

68. Ganda OP, Srikanta S, Gleason RE, Soeldner JS, Eisenbarth GS. Diminished A cell secretion in the early phase of Type I diabetes mellitus. Metabolism 1986; 35:1074-1077.

69. Rabinowe SL, Larsen PR, Antman EM, George KL, Friedman PL, Jackson RA, Eisenbarth GS. Amiodarone therapy and autoimmune thyroid disease: elevation of a new monoclonal antibody defined T cell subset. Am J Med 1986; 81:53-57.

70. Eisenbarth GS, Srikanta S, Rabinowe SL, Jackson RA, Ganda OP, Soeldner JS. Restoration of first phase insulin secretion by daily prednisone in two islet cell antibody positive non-diabetic individuals. Trans Proceed 1986; 18:805-808.

10

ORIGINAL REPORTS (Contd.)

71. Hattori M, Buse JB, Jackson RA, Glimcher L, Makino S, Moriwaki K, Dorff M, Minami M, Kuzuya H. Imura H. Seidman JG, Eisenbarth GS. The NOD mouse: recessive diabetogenic gene within the major histo-compatibility complex. Science 1986; 231:733-735.

72. Srikanta S, Ricker AT, McCulloch DK, Soeldner JS, Eisenbarth GS, Palmer JP. Autoimmunity to insulin, beta cell dysfunction and development of insulin dependent diabetes mellitus. Diabetes 1986; 35:139-142.

73. Rabinowe SL, George KL, Laughlin R, Soeldner JS, Eisenbarth GS. Congenital rubella: monoclonal antibody defined T cell abnormalities in young children. Am J Med 1986; 81:779-782.

74. Srikanta S. Krisch K. Eisenbarth GS. Islet cell protein defined by monoclonal islet cell antibody HISL-19. Diabetes 1986; 35:300-305.

75. Dib S, Vardi P, Connelly J, Eisenbarth GS, Soeldner JS. Immune changes associated with insulin dependent diabetes may remit without causing the disease: a study in identical twins. Br Med J 1986; 292:1670.

76. Posillico JT, Wortsman J, Srikanta S, Eisenbarth GS, Malette L, Brown EM. Parathyroid cell surface autoantibodies that inhibit parathyroid hormone secretion from dispersed human parathyroid cells. Bone and Mineral Research 1986; 5:475-485.

77. Kaye WA, Adri MNS, Soeldner JS, Rabinowe SL, Kahn CR, Bistrian B, Srikanta S, Ganda OP, Eisenbarth GS. Acquired interleukin-2 production defect in patients with Type I diabetes mellitus. N Engl J Med 1986; 315:920-924.

78. Srikanta S, Telen M, Posillico JT, Dolinar R, Krisch K, Haynes BF, Eisenbarth GS. Monoclonal antibodies to a human islet cell surface glycoprotein: 4F2 and LC7-2. Endocrinology 1987; 120:2240-2244.

79. Posillico JT, Srikanta S, Eisenbarth GS, Quaranta V, Kajiji S, Brown EM. Binding of monoclonal antibody (4F2) to its cell surface antigen on dispersed adenomatous parathyroid cells raises cytosolic calcium and inhibits parathyroid secretion. J Clin Endo Metab 1987; 64:43-50.

80. Rabinowe SL, Nayak RC, Krisch K, George KL, Eisenbarth GS, Aging in man: linear increase of a novel T cell subset defined by anti-ganglioside monoclonal 3G5. J Exp Med 1987; 165:1436-1441.

ORIGINAL REPORTS (Contd.)

81. Dib SA, Colman PG, Dotta F, Tautkus M, Rabizadeh A, Eisenbarth GS. Expression of "cytoplasmic" islet cell antigens by rat pancreas. Diabetes 1987; 36:982-985.

82. Vardi P, Dib SA, Tuttleman M. Connelly JE, Grinbergs M, Rabizadeh A, Riley WJ, Maclaren NK, Eisenbarth GS, Soeldner JS. Competitive insulin autoantibody RIA: prospective evaluation of subjects at high risk for development of Type I diabetes mellitus. Diabetes 1987;36:1286-1291.

83. Baekkeskov S, Landan M, Kristensen JK, Srikanta S, Bruining GJ, Mandrup-Poulsen T, Beaufort C, Soeldner JS, Eisenbarth GS, Lindgren F, Sundquist G, Lernmark A. Antibodies to a 64,000 Mr human islet cell antigen precede the clinical onset of insulin dependent diabetes. J Clin Invest 1987; 79:926-934.

84. Omar MAK, Srikanta S, Eisenbarth GS. Human islet cell antibodies: immunoglobulin class and subclass distribution defined by monoclonal antibodies. Diabetes Res 1987; 4:155-157.

85. Karp M, Rabizadeh A. Eisenbarth GS. Prolonged sera incubation to improve sensitivity of double-immunofluorescent monoclonal BISL-32 FITC protein-A assay. Diabetes Care 1987; 10:535-536.

86. Posillico JT, Wilson RE, Srikanta S, Eisenbarth GS, Letarte M, Quackenbush EJ, Quaranta V, Kajaji S, Brown EM. Monoclonal antibody mediated modulation of parathyroid hormone secretion by dispersed parathyroid cells. Arch Surg 1987; 122(4):436-442.

87. Dib SA, Vardi P. Bonner-Weir S, Eisenbarth GS. Selective localization of Factor VIII. Antigenicity to endothelial cells and expression of class II antigens by normal human pancreatic ductal epithelium. Diabetes 1988; 37:482-487.

88. Ikegami H, Makino S, Harada M, Eisenbarth GS, Hattori M. The CTS mouse, a sister strain of the NOD: similar class II but different class I gene products. Diabetologia 1988; 31:254-258.

89. DiMario U, Dotta F, Crisa L, Anastasi E, Andreani D, Dib S, Eisenbarth GS. Circulating anti-immunoglobulin antibodies in recent onset Type I diabetic patients. Diabetes 1988; 37:462-466.

90. Colman PG, Nayak RC, Campbell IL, Eisenbarth GS. Binding of cytoplasmic islet cell antibodies is blocked by human pancreatic glycolipid extracts. Diabetes 1988; 37:645-652.

91. Dotta F, Nayak RC, Dib SA, DiBella E, Krisch K, Posillico JT, Ricker AT, Di Mario U, Eisenbarth GS. A novel neuroendocrine cell surface glycoprotein: identification, isolation and initial characterization. Endocrinology 1988; 122:1263-1268.

..G.S.Eisenbarth,M.d.,Ph.D.

ORIGINAL REPORTS (Contd.)

92. Nayak RC, Berman AB, George KL, Eisenbarth GS, King GL. A monoclonal antibody (3G5) defined ganglioside antigen is expressed on the cell surface of microvascular pericytes. J Exp Med 1988; 167:1003-1015.

93. Kelly VE, Gaulton GN, Hattori M, Ikegami H, Eisenbarth GS, Strom TB. Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. J Immunol 1988; 140:59-61.

94. Colman PG, DiMarlo U, Rabizadeh A, Dotta F, Anastasi E, Eisenbarth GS. A prozone phenomenon interferes in islet cell antibody detection: direct comparison of two methods in subjects at risk to develop diabetes and in insulin dependent diabetes at onset. J Autoimmunity 1988; 1:109-117.

95. Colman PG, Tautkus M, Rabizadeh A, Cahill C, Eisenbarth GS. Assay for islet cell antibodies with rat pancreas and peroxidase protein A. Diabetes Care 1988; 11:367-368.

96. Vardi P, Ziegler AG, Matthews JH, Dib S, Keller RJ, Ricker AT, Wolfsdorf JI, Herskowitz RD, Rabizadeh A, Eisenbarth GS, Soeldner JS. Concentration of insulin autoantibodies at onset of Type I diabetes: inverse log-linear correlation with age. Diabetes Care 1988; 11:736-739.

97. Herskowitz RD, Wolfsdorf JI, Ricker AT, Vardi P, Dib S, Soeldner JS, Eisenbarth GS. Transient hyperglycemia in childhood: identification of a subgroup with imminent diabetes mellitus. Diabetes Res 1988; 9:161-167.

98. Jackson RA, Soeldner JS, and Eisenbarth GS. Predicting insulin-dependent diabetes. Lancet 1988; Sept 10, 627.

99. Ziegler AG, Vardi P, Ricker AT, Hattori M, Soeldner JS, Eisenbarth GS. Radioassay determination of insulin autoantibodies in NOD mice: correlation with increased risk of progression to overt diabetes. Diabetes 1989; 38:358-363.

100. Rabinowe SL, Rubin L, George KL, Adri MNS, Eisenbarth GS. Trisomy 21 (Down's syndrome): autoimmunity, aging and monoclonal antibody defined T cell abnormalities. J Autoimmunity 1989; 2:25-30.

101. Ziegler AG, Vardi P, Gross DJ, Bonner-Weir S, Villa-Kamaroff L, Halban P, Ikegami H, Soeldner JS, Eisenbarth GS. Production of insulin antibodies by mice rejecting insulin transfected cells. J Autoimmunity 1989; 2:219-227.

102. Herskowitz RD, Jackson RA, Soeldner JS, Eisenbarth GS. Pilot trial to prevent Type I diabetes: progression to overt IDDM despite oral nicotinamide. J of Autoimmunity 1989;2:733-737.

ORIGINAL REPORTS (Contd.)

103. Ziegler AG, Ziegler R, Vardi P, Jackson RA, Soeldner JS, Eisenbarth GS. Life table analysis of progression to diabetes of anti-insulin autoantibody-positive relatives of individuals with Type I diabetes. Diabetes 38:1320-1325, 1989.

104. Dotta F, Colman PG, Nayak RC, Lombardi D, Scharp DW, Andreani D, Pontieri GM, Di Mario U, Lenti L, Eisenbarth GS. Ganglioside expression in human pancreatic islets. Diabetes 38:1478-1483. 1989.

105. Faustman D, Eisenbarth G, Daley J, Breitmeyer J. Abnormal T-lymphocyte subsets in Type I diabetes. Diabetes 38:1462-1467, 1989.

106. Gardner SL, Dotta F, Nayak RC, George KL, Eisenbarth GS, Halban PA. Modulation of sodium butyrate of the differentrated status of a clonal pancreatic B-cell line (RIN). Diabetes Research 1989, 12:93-99.

107. Bleich D, Jackson RA, Soeldner JS, Eisenbarth GS. Analysis of metabolic progression to Type I diabetes in islet cell antibody positive relatives of patients with Type I diabetes. Diabetes Care 13:111-118, 1990.

108. Ikegami H, Eisenbarth GS, Hattori M. MHC-linked diabetogenic gene of the NOD mouse: analysis of genomic DNA amplified by polymerase chain reaction. J Clin Invest 85:18-24, 1990.

109. Ziegler R, Alper CA, Awdeh ZL, Castano L, Brink SJ, Soeldner S, Jackson RA, Eisenbarth G S. Specific association of HLA-DR4 with increased prevalence and level of insulin autoantibodies in first degree relatives of patients with type I diabetes. Diabetes 1991; 40:709-714.

110. Vardi P, Crisa L, Jackson RA, Herskowitz RD, Wolfsdorf JI, Einhorn D, Linarelli L, Dolinar R, Wentworth S, Brink SJ, Starkman H, Soeldner JS, Eisenbarth GS. Predictive value of intravenous glucose tolerance test insulin secretion less than or greater than the first percentile in islet cell antibody positive relatives of type I (insulin-dependent) diabetic patients. Diabetologia 1991; 34:93-102.

111. Erlich HA, Griffith RL, Bugawan TL, Ziegler R, Alper C, Eisenbarth GS. Implication of specific DQB1 alleles in genetic susceptibility and resistance by identification of IDDM siblings with novel HLA-DQB1 allele and unusual DR2 and DR1 haplotypes. Diabetes 1991; 40:478-481.

....*G.S.Eisenbarth, M.d., Ph.D.*

ORIGINAL REPORTS (Contd.)

112. Castano L, Russo E, Zhou L, Lipes MA, Eisenbarth GS. Identification and cloning of a granule autoantigen (carboxypeptidase H) associated with type I diabetes. J Clin Endo Metabl, Vol 73:1197-1201, 1991.

113. Zhang SJ, Davidson L, Eisenbarth G, and Weiner HL. Suppression of Diabetes in NOD Mice by Oral Administration of Porcine Insulin. P.N.A.S., August 1991.

114. Gianani R, Pugliese A, Bonner-Weir S, Shiffrin AJ, Soeldner JS, Erlich H, Awdeh Z, Alper CA, Jackson RA and Eisenbarth GS. Prognostically significant heterogeneity of cytoplasmic islet cell antibodies in relatives of patients with type I diabetes. In press, Diabetes March 1991.

115. Faustman D, Guo J, Lin H, Fu Y, Ziljlstra M, Eisenbarth G, Jaenish R, Avruch J, and Li X. Linkage of faulty MHC class I to autoimmune diabetes. Science Vol 254:1756-1761, 1991

116. Simon S, Awdeh Z, Campbell RD, Ronco II P, Brink SJ, Eisenbarth GS, Yunis EJ, Alper CA. A restriction fragment of the C2 gene is a unique marker for C2 deficiency and the uncommon C2 Allele C2*B (a marker for type I diabetes). JCI 88:2142-2145, 1991.

C H A P T E R S , R E V I E W S , S Y M P O S I U M S , E T C.

1. Lebovitz HE, Eisenbarth GS. Hormonal control of cartilage metabolism. In: Vitamins and Hormones 1975; 33:575.

2. Eisenbarth GS, Lebovitz HE. Immunogenetics of the polyglandular failure syndrome. Life Sciences 1978; 22:1675-1684.

3. Walsh F, Eisenbarth GS. Preparation of monoclonal antibodies to chick neural retina cell surface antigens. Biochem Soc Trans 1979; 7:1016-1018.

4. Haynes BF, Hemler ME, Mann DL, Eisenbarth GS, Strominger JL, Fauci AS. Characterization of a monoclonal antibody (4F2) which binds to human monocytes and a subset of activated lymphocytes. In: Heterogeneity of Mononuclear Phagocytes. Foster O, Landry M (eds), London, Academic Press, 1981; 53-59.

5. Eisenbarth GS. Monoclonal antibodies to islet T lymphocyte and neuronal cell surface differentiation antigens. In: Monoclonal Antibodies in Endocrine Research. Fellows R, Eisenbarth GS (eds), New York, Raven Press, 1981; 33-39.

6. Eisenbarth GS. Application of monoclonal antibody techniques to biochemical research. Analytical Biochem 1981; 111:1-6.

7. Eisenbarth GS, Jackson R. Immunogenetics of polyglandular failure and related diseases. In: HLA and Endocrine Disease. Farid N (ed), New York, Academic Press, 1981; 235-264.

8. Eisenbarth GS, Shimizu K, Conn M, Mittler R, Wells S. Monoclonal antibody F12 A2B5: expression on neuronal and endocrine cells. In: Monoclonal Antibodies to Neuronal Antigens. Cold Spring Harbor Symposium 1981; 209-218.

9. Fellows R, Eisenbarth GS (eds). Moroclonal Antibodies in Endocrine Research. New York, Raven Press, 1981.

10. Eisenbarth GS, Jackson R. Application of monoclonal antibody techniques to endocrinology. Endocrine Reviews 1982; 3:26-39.

11. Buse JB, Rowley RF, Eisenbarth GS. Disordered cellular immunity in Type I diabetes of man and the BB rat. Surv Immunol Res 1982; 1:339-351.

12. Eisenbarth GS, Rassi N. The polyglandular failure syndromes. In: Autoimmune Endocrine Disease. Davies TF (ed), New York, John Wiley & Sons, Inc., 1983; 193-206.

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

13. Srikanta S, Eisenbarth GS. Case Report: Type I diabetes. In: Diagnostic Immunology, a Compendium of Case Studies. Escobar MR, Virella G (eds), New York, Plenum Publishing Corp., 1983.

14. Eisenbarth GS. Immunotherapy of Type I diabetes. Diabetes Care 1983; 6:521-523.

15. Scearce RM, Eisenbarth GS. Production of monoclonal antibodies reacting with the cytoplasm and surface of differentiated cells. In: Methods in Enzymology: Neuroendocrine Peptides. Colowick SP, Kaplan NO (eds), Academic Press, 1983; 103:459-469.

16. Eisenbarth GS, Jackson RA, Srikanta S. Type I diabetes: autoimmunity and immunodeficiency. In: Monoclonal Antibodies: Probes for the Study of Autoimmunity and Immunodeficiency. Haynes BF, Eisenbarth GS (eds). Academic Press, 1983; 197-219.

17. Haynes BF, Eisenbarth GS. Use of monoclonal antibodies to identify cell surface antigens of human neuroendocrine thymic epithelium. In: Monoclonal Antibodies: Probes for the Study of Autoimmunity and Immunodeficiency. Haynes BF, Eisenbarth GS (eds), Academic Press, 1983; 47-65.

18. Haynes BF, Warren RW, Buckley HR, McClure JE, Goldstein AL, Henderson FW, Hensley LL, Eisenbarth GS. Demonstration of abnormalities in expression of thymic epithelial surface antigens in surface cellular immunodeficiency diseases. In: Birth Defects: Original Article Series 1983; 130:1182-1188.

19. Eisenbarth GS. Type I diabetes mellitus: prediction and hopes of prevention. Juvenile Diabetes Foundation Countdown, 1983; 6:14-15.

20. Jackson RA, Eisenbarth GS. Type I diabetes of man and the BB rat: monoclonal antibody-defined T cell abnormalities. Diagnostic Immunol 1983; 1:240-244.

21. Eisenbarth GS, Mark EJ. Case records of the Massachusetts General Hospital: a 64 year old man with hypoglycemia and thoracic mass. N Engl J Med 1983; 310:580-587.

22. Eisenbarth GS. The immunoendocrinopathy syndromes. In: Williams Textbook of Endocrinology. Saunders Publishing, 1984.

23. Eisenbarth GS. Monoclonal antibody "biotechnology". In: Comprehensive Biotechnology. Robinson CW (ed), 1984.

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

24. Eisenbarth GS, Jackson RA, Srikanta S. Powers AC, Buse JB, Rabizadeh A, Mori H. Utilization of monoclonal antibody techniques to study Type I diabetes mellitus. In: Immunology in Diabetes 1984. Andreani D, DiMario U, Federlin KF, Heding LG (eds), Edinburgh, London, Kimpton Medical Publications. 1984; 143-157.

25. Kaldany A, Busick EJ, Eisenbarth GS. Diabetes mellitus and the immune system. In: Joslin's Diabetes Mellitus: 12th Edition. Marble A, Krall LP, Bradley RF, Christlieb AR, Soeldner JS (eds), Philadelphia, Lea and Febinger Publishing, 1984.

26. Rabinowe SL, Eisenbarth GS. Type I diabetes mellitus: a chronic autoimmune disease. Ped Clin NA, 1984.

27. Eisenbarth GS. Autoimmune beta cell insufficiency. Triangle 1984; 23:111-124.

28. Srikanta S, Eisenbarth GS. Anti-islet cell monoclonal antibodies. In: Methods in Diabetes Research. Larner J, Pohl S (eds). John Wiley & Sons, 1984.

29. Rabinowe SL, Eisenbarth GS. Immunotherapy of Type I (insulin dependent) diabetes mellitus. In: Immunology in Diabetes. Andreani D, DiMario U, Federlin KF, Heding LG (eds), London, Kimpton Medical Publications 1984; 171-175.

30. Srikanta S, Eisenbarth GS. Anti-islet cell monoclonal antibodies. In: Methods in Diabetes Research. Larner J, Pohl SL (eds), New York, John Wiley & Sons, Inc., 1985; 195-208.

31. Powers AC, Eisenbarth GS. Autoimmunity to islet cells in diabetes mellitus. Advances in Internal Medicine 1985; 36:533-544.

32. Rabinowe SL, Srikanta S, Jackson RA, Nayak RC, Ganda OP, Soeldner JS, Eisenbarth GS. "Prediction" of overt Type I diabetes and pilot trials of immunomodulation. Serrano-Rios M, Lefebvre (eds), Excerpta Medica, Proceedings of the 12th Congress of the International Diabetes Federation, Madrid, Spain, September 23-28, 1985. Diabetes.

33. Eisenbarth GS. Immunotherapy of Type I diabetes. Ann Int Med 1985; 102:845-847.

34. Rabinowe SL, Eisenbarth GS. Polyglandular autoimmunity. Adv Int Med 1986; 31:293-307.

35. Buse JB, Eisenbarth GS. Autoimmune endocrine disease. Vitamins and Hormones 1986; 42:253-314.

36. Eisenbarth GS. Type I diabetes mellitus. A Chronic autoimmune disease. N Engl J Med 1986; 314:1360-1368.

....*G.S.Eisenbarth,M.d.,Ph.D.*

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

37. Eisenbarth GS. Islet cell antibodies. Laboratory Management 1986; 24:23-28.

38. Srikanta S, Eisenbarth GS. Islet cell antigens. Molecular Biol Med 1986; 3:113-127.

39. Eisenbarth GS, Jackson RA, Srikanta S, Nayak RC, Rabinowe SL, Posillico JT, Brown EM, Ikegami H, Hattori M. Organ specific autoimmunity: T cells and target cells probed with monoclonal antibodies. In: Monoclonal Antibodies: Basic Principles, Experimental and Clinical Applications in Endocrinology. Forti G, Seri M, Lipsett MB (eds). New York, Raven Press, 1986; 15-25.

40. Eisenbarth GS. Immunogenetics of endocrine disorders. immunology Today 1986; 6(12):345-376.

41. Eisenbarth GS. Prediction, prevention and cure: the promise of new immunological insights. Juvenile Diabetes Foundation Countdown 1986, November.

42. Colman PG, Nayak RC, Connelly J, Rabizadeh A, Soeldner JS, Eisenbarth GS. Islet cell antibodies: clinical utility and target antigen(s). In: The Immunology of Diabetes Mellitus. Jaworski MA, Molner GD, Ragotti RV, Singh B (eds). Excerpta Medica, Amsterdam. Elsevier Science Publishers, Netherlands, 1986; 345-350.

43. Ricker AT, Stockberger SM, Halban PA, Eisenbarth GS, Bonner-Weir S. Hyperimmune response to microencapsulated xenogeneic tissue in non-obese diabetic mice. In: The Immunology of Diabetes Mellitus. Jaworski MA, Molnar GD, Rajotti RV, Singh B (eds). Excerpta Medica, Amsterdam. Elsevier Science Publishers, Netherlands. 1986; 193-200.

44. Brink SJ, Fleischnick E, Srikanta S, Jackson RA, Ganda OP, Yunis E, Alper C, Soeldner JS, Eisenbarth GS. Pre-Type I diabetes mellitus detected in a screening program. Ped Adolesc Endocrinol 1986; 15:54-60.

45. Soeldner JS, Srikanta S, Eisenbarth GS, Gleason RE. Pre-hyperglycemic diabetes mellitus. Clin Chem 1986; 32:B7-B18.

46. Eisenbarth GS. Type I diabetes mellitus - a chronic autoimmune disease. N Engl J MEd 1986; 314:1360-68.

47. Bhatia E, Eisenbarth GS. Pathogenesis of Type I diabetes: analogy to a plaque assay. Forums in Immunology 1986. Elsevier Ann Inst Pasteur/ Immunol, 1986; 137D:228-231.

....*G.S.Eisenbarth,M.d.,Ph.D.*

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

48. Baekkeskov S, Bruining J, Srikanta S, Mandrup-Poulsen T, DeBeaufort C, Elsenbarth GS, Nerup J, Lernmark A. Antibodies to a Mr 64,000 human islet cell protein in the prediabetic period of IDDM patients. Ann NY Acad Sci 1986; 415-417.

49. Assan R, Bach JF, Czernichow P, Dupre J, Stiller CR, Eisenbarth GS, Gries FA, Kolb H, Harrison LC, Lafferty K. Immunosuppressive drugs in diabetes. Lancet 1986; Nov:1097.

50. Nayak RC, Rabizadeh A, Srikanta S, Eisenbarth GS, Spitalnik S. Islet cell autoantigen(s) in Type I diabetes mellitus: preliminary biochemical characterization. The Second International Conference on the Use of Human Tissues and Organs for Research and Transplant. 1986; 30-39.

51. Eisenbarth GS, Connelly J, Soeldner JS. The "natural" history of Type I diabetes. Diabetes/Metabolism Reviews 1987; 873-891.

52. Colman PG, Eisenbarth GS. Immunological approaches to the treatment and prevention of childhood diabetes. Postgrad Med 1987; 81:146-160.

53. Eisenbarth GS. Lilly Lecture: Genes, generator of diversity, glycoconjugates and autoimmune beta cell insufficiency in Type I diabetes. Diabetes 1987; 36:355-364.

54. Eisenbarth GS. Predicting Type I diabetes. Clin Chemistry, The Hague, Netherlands, July 1987; in press.

55. Colman PG, Eisenbarth GS. Immunotherapy in Type I diabetes. Approaches to prevention and treatment Postgrad Med 1987; 81:145-155.

56. Eisenbarth GS. Type I diabetes: clinical implications of autoimmunity. Hospital Practice 1987; 22:71,174,177.

57. Colman PG, Eisenbarth GS. T cell abnormalities in Type I diabetes of man. Folia Allergol Immunol Clin 1987; 34:29-38.

58. Rabinowe SL, Eisenbarth GS. Immunology of Type I diabetes mellitus. Brink SJ (ed), 1988; in press.

59. Eisenbarth GS. Immune endocrine disorders. In: Manual of Endocrinology and Metabolism, Lavin N (ed), 1988; in press.

60. Eisenbarth GS. Autoimmune endocrine disorders. In: DeGroot's Textbook of Endocrinology, Second Edition, 1988; in press.

20

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

61. Nayak RC, Colman PG, Eisenbarth GS. How are monoclonal antibodies related to autoimmune serology? Clin in Immunol and Allergy. Doniach D, Bottazzo GF (eds). 1988; in press.

62. Weir GC, Eisenbarth GS. Etiology of diabetes. Chi Zhi-Sheng (ed). Diabetology, China, 1988, in press.

63. Eisenbarth GS. Type I diabetes. Textbook of Internal Medicine, Kelly WN (ed), JB Lippincott Co., Philadelphia, PA. 1988; in press.

64. Eisenbarth GS, Vardi P, Ziegler AG, Colman PG, Nayak RC, Dotta F, Jackson RA, Fujii S, Ricker AT, Rabinowe SL, Ikegami H, Soeldner JS, Hattori M. Lessons from the NOD mouse and BB rat: similarities and contrasts. In: Frontiers in Diabetes Research; Lessons from Animal Diabetes: II, Renold AE, Shafir E (eds). John Libbey & Co. Ltd., London, 1988; in press.

65. Eisenbarth GS, Kahn CR. Diabetes mellitus and the non-diabetic mellituria: etiology and pathogenesis. In: Text of Endocrinology, Becker (ed), 1988; in press.

66. Wortsman J, Posillico JT, Brown E, Srikanta S, Eisenbarth GS, Mallette LE. Letter. N Engl J Med 1988; in press.

67. Eisenbarth GS. Type I diabetes. Textbook of Internal Medicine. Kelley WN (ed), J.B. Lippincott Co. 1988.

68. Colman PG, Eisenbarth GS. Immunology of Type I diabetes 1987. Diabetes Annual 4, Alberti KG, Krall KP (eds), Elsevier Science Publishers, Amsterdam, 1988; 17-55.

69. Eisenbarth GS. Predicting Type I diabetes. In: The World Book of Diabetes in Practice. Volume 3. Krall LP, Alberti KGMM, Turtle J (eds). Elsevier Science Publishers, 1988; 16-20

70. Keller RJ, Eisenbarth GS. "The immunopathogenesis of Type I diabetes mellitus", In: Immunotherapy of Type I Diabetes and Selected Autoimmune Disorders. Eisenbarth GS (ed), CRC Press, Inc., Boca Raton FL, 1989; 1-21.

71. Jackson RA, Eisenbarth GS. Autoimmune polyglandular syndromes. In: Textbook of Internal Medicine. Kelly WN (ed), JP Lippincott Co., Philadelphia, PA, 1988; in press.

72. Eisenbarth GS, Nayak RC, Rabinowe SL. Type I diabetes as a chronic autoimmune disease. Journal of Diabetic Complications 1988 2:54-58.

....*G.S.Eisenbarth,M.d.,Ph.D.*
CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

73. Dotta F, Eisenbarth GS. Type I diabetes mellitus: a predictable autoimmune disease with inter-individual variation in the rate of beta cell destruction. Clin Immunol Immunopath 1988: 50:585-595.

74. Lipes M, Wilson K, Eisenbarth GS. Predicting and Monitoring the Rate of Autoimmune Beta Cell Destruction. In: Progress in Endocrinology 1988. Imura H, Shimizu K, Yoshida S (eds), Excerpta Medica International Congress Series 799; 1:649-664.

75. Ziegler AG, Jackson RA, Soeldner JS, Eisenbarth GS. Humoral markers of Type I diabetes: predicting Type I diabetes. In: Diabetes 1988 Proceedings of the 13th Congress of the International Diabetes Federation, Sydney, Australia, Larkins RE, Zimment PZ, Chisolm DJ (eds), November 20-25, 1988.

76. Eisenbarth GS. Predicting Type I diabetes (a dual parameter linear model). In: World Book of Diabetes in Practice. Elsevier Science Publishers, Krall LP (ed) 1988.

77. Jackson RA, Eisenbarth GS. Autoimmune endocrine disorders. In: Textbook of Internal Medicine. Kelley WN (ed), J.B. Lippincott Co., 1988; in press.

78. Eisenbarth GS. Type I diabetes mellitus: a chronic and predictable autoimmune disease. In: Current Concepts. A Scope publication, Upjohn. 1989.

79. Zielger AG, Ziegler R, Jackson RA, Eisenbarth GS. Type I diabetes: testing the "linear" destruction hypothesis: The Joslin study. In: Immunotherapy of Type I diabetes. Andreani D, Kolb H. Pozzilli P (eds), John Wiley and Sons Ltd, 1989; 155-167.

80. Zielger R, Jackson RA, Ziegler AG, Eisenbarth GS. Type I diabetes: polygenic inheritance, multiple autoantigens and dual parameter prediction. J. of Autoimmunity 3:69-74, 1990.

81. Zielasek J, Jackson RA, Eisenbarth GS. The potentially simple mathematics of Type I diabetes. Clin Immunol and Immunopath 1989;52:347-365.

82. Eisenbarth GS, Jackson RA. The immunoendocrinopathy syndromes. In: Williams Textbook of Endocrinology. In press.

83. Russo E, Jackson RA, Dotta F, Lipes MA, Castano L, Zielasek J, Bleich D, Keller RJ, Ziegler R, Hattori M, Nayak RC, Herskowitz RD. Type I diabetes as a "Mendelian" and "regulated" immune process. In: The Molecular Biology of Autoimmune Disease, edited by A.G. Demaine et al. NATO ASI Series, Vol H 38, Springer-Verlag Berline Heidelberg 1990.

CHAPTERS, REVIEWS, SYMPOSIUMS, ETC (Contd.)

84. Ziegler AG, Herskowitz RD, Jackson RA, Soeldner S, Eisenbarth GS. Predicting Type I diabetes. Diabetes Care 13:762-775, 1990

85. Ziegler AG, Eisenbarth GS. Immunology of IDDM (Type I diabetes) - 1989. In: The Diabetes Annual/5. K.G.M.M. Alberti and LP Krall (eds). 1990 Elsevier Science Publisher B.V. 22-50.

86. Ziegler R, Eisenbarth GS. Multiple target-antigens in pre-Type I diabetes: implications for prediction. Hormone Research 33:144-151, 1990.

87. Castano L, Eisenbarth GS. Type I diabetes: a chronic autoimmune disease of man, mouse and rat. Ann Rev Immunol 8:647-679, 1990.

88. Wilson K, Eisenbarth GS. Immunopathogenesis and immunotherapy of Type I diabetes. Ann Rev Med 1990 41:497-508.

89. Lipes M, Eisenbarth GS. Transgenic Mouse Models of Type I Diabetes. Diabetes, 1990 39:879-884.

90. Eisenbarth GS, Jackson RA, Ziegler R, Castano L, Bleich D, and Dotta F. Prediction/Prevention of Type I Diabetes, Proceedings of the II Workshop Lilly Editrice Kurtis IL Diabetes, in press.

91. Bhimji S and Eisenbarth GS. Type I diabetes mellitus and autoimmunity. Annals RCPSC 1990 23:531-536.

92. Eisenbarth GS. Type I diabetes in 2nd edition of textbook of Internal Medicine. W Kelley ed. JB Lippincott Co. 1990.

93. Dotta F and Eisenbarth GS. Aetiopathogenesis of type I diabetes in western society in International Textbook of Diabetes Mellitus eds. Kg Alberti, RA DeFronzo, H Keen, and P Zimmet Wiley Medical Publication. In Press.

94. Eisenbarth GS. Autoimmune seriology in type I diabetes and the prediabetic state. IDF Bulletin Volume XXXV Number 2.

95. Eisenbarth GS. Clinical prediction of type I diabetes. Horizons In Medicine 3, In Press, 1991

96. Pietropaolo M, Eisenbarth G. Immunotherapy of Type I Diabetes. Current Therapy in Allergy, Immunology, and Rheumatology, In Press 1991.

APPENDIX A

Gly-Leu-Leu-Glu-Cys-Cys-Ala-Arg-Cys-Leu-Val-Gly-Ala-Pro-Phe-Ala-Ser-Leu-Val-Ala- 20

Thr-Gly-Leu-Cys-Phe-Phe-Gly-Val-Ala-Leu-Phe-Cys-Gly-Cys-Gly-His-Glu-Ala-Leu-Thr- 40

Gly-Thr-Glu-Lys-Leu-Ile-Glu-Thr-Tyr-Phe-Ser-Lys-Asn-Tyr-Gln-Asp-Tyr-Glu-Tyr-Leu- 60

Ile-Asn-Val-Ile-His-Ala-Phe-Gln-Tyr-Val-Ile-Tyr-Gly-Thr-Ala-Ser-Phe-Phe-Phe-Leu- 80

Tyr-Gly-Ala-Leu-Leu-Leu-Ala-Tyr-Gly-Phe-Tyr-Thr-Thr-Gly-Ala-Val-Arg-Gln-Ile-Phe- 100

Gly-Asp-Tyr-Lys-Thr-Thr-Ile-Cys-Gly-Lys-Gly-Leu-Ser-Ala-Thr-Val-Thr-Gly-Gly-Gln- 120

Lys-Gly-Arg-Gly-Ser-Arg-Gly-Gln-His-Gln-Ala-His-Ser-Leu-Glu-Arg-Val-Cys-His-Cys- 140

Leu-Gly-Lys-Trp-Leu-Gly-His-Pro-Asp-Lys-Phe-Val-Gly-Ile-Thr-Tyr-Ala-Leu-Thr-Val- 160

Val-Trp-Leu-Leu-Val-Phe-Ala-Cys-Ser-Ala-Val-Pro-Val-Tyr-Ile-Tyr-Phe-Asn-Thr-Trp- 180

Thr-Thr-Cys-Gln-Ser-Ile-Ala-Ala-Pro-Ser-Lys-Thr-Ser-Ala-Ser-Ile-Gly-Thr-Leu-Cys- 200

Ala-Asp-Ala-Arg-Met-Tyr-Gly-Val-Leu-Pro-Trp-Asn-Ala-Phe-Pro-Gly-Lys-Val-Cys-Gly- 220

Ser-Asn-Leu-Leu-Ser-Ile-Cys-Lys-Thr-Ala-Glu-Phe-Gln-Met-Thr-Phe-His-Leu-Phe-Ile- 240

Ala-Ala-Phe-Val-Gly-Ala-Ala-Ala-Thr-Leu-Val-Ser-Leu-Val-Thr-Phe-Met-Ile-Ala-Ala- 260

Thr-Tyr-Asn-Phe-Ala-Val-Leu-Lys-Leu-Met-Gly-Arg-Gly-Thr-Lys-Phe 270

Sequence of bovine PLP.

INSULIN FRAGMENTS

10 - 18

1 - 21

(A1) H-GIVEQCCTSICSLYQLENYCN-OH (A21)

(B1) H-FVNQHLCGSHLVEALYLVCGERGFFYTPKT-OH (B30)

1 - 30

1 - 12

23 - 30

10 - 22

11 - 30

Amino acid sequence alignment of myelin basic proteins

Position markers: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170

Species labels (in order for each block): Hum, Bov, Rab, GPig, RatS, Chic

Block 1 (residues 1–30):
```
Hum   A S Q K R P S Q R ... G S K Y L A T A S T M D H A R H G F L P R H R D T
Bov   A A Q K R P S Q R - - S K Y L A S A S T H D H A R H G F L P R H R D T
Rab   A S Q K R P S Q R H G S K Y L A T A S T H D H A R H G F L P R H R D T
GPig  A S Q K R P S Q R H G S K Y L A T A S T H D H A R H G F L P R H R D T
RatS  A S Q K R P S Q R H G S K Y L A T A S T H D H A R H G F L P R H R D T
Chic  A S Q K R S S P R H G S K - H A S A T S T D H A R H G S - P R H R D S
```

Block 2 (residues 40–70):
```
Hum   G I L D S I G R F F G G D R G A P K R G S G K D S H H P A R T A H Y G
Bov   G I L D S L G R F F G S D R G A P K R G S G K D S H H P A R T A H Y G
Rab   G I L D S I G R F F S D R G A P K R G S G K D - - H A A R T T H Y G
GPig  G I L D S I G R F F G S D R A A P K R G S G K D S H H A A R T T H Y G
RatS  G I L D S I G R F F S G D R G A P K R G S G K D S H H A A R T T H Y G
Chic  G L L D S L G R F F G G D R - V P K R G F G K D - - - A A R A S H H V G
```

Block 3 (residues 80–100):
```
Hum   S L P Q K S - H G R T Q D E H P V V H F F K H I V T P R T P P P S Q G
Bov   S L P Q K A Q H G R P Q D E H P V V H F F K H I V T P R T P P P S Q G
Rab   S L P Q K S - H G R P Q D E H P V V H F F K H I V T P R T P P P S Q C
GPig  S L P Q K S Q - - R S Q D E H P V V H F F K H I V T P R T P P P S Q C
RatS  S L P Q K S Q - - R T Q D E H P V V H F F K H I V T P R T P P P S Q C
Chic  S I P Q R S Q H - R F(H,D,G,H) P V V H F F K H I V S P R T P P P H Q A
```

Block 4 (residues 110–140):
```
Hum   K G R G L S L S R F S W G A E G Q R P G F G Y G G R A S D Y K S A H K
Bov   K G R G L S L S R F S W G A E G Q R P G F G Y G G R A S D Y K S A H K
Rab   K G R G T V L S R F S W G A E G Q K P G F G Y G G R A A D Y K S A H K
GPig  K G R G L S L S R F S W G A E G Q K P G F G Y G G R A - D Y K S - H K
RatS  K G R G L S L S R F S W - - - - - - - - - - - - - - - - - - - K
Chic  K G R G L S L T R F S W G S E G H K P G S G Y G S K F T E H S A H K
```

Block 5 (residues 150–177):
```
Hum   G F K G - V - D A Q G T L S K I F K L G G R - - - - D S R S G S P H A R R
Bov   G L K G - - H D A Q G T L S K I F K L G G R - - - - D S R S G S P H A R R
Rab   G L K G - A - D A Q G T L S R L F K L G G R - - - - D S R S G S P H A R R
GPig  G F K G - A H D A Q G T L S K I F K L G G R - - - - D S R S G S P H A R R
RatS  - - - - - - - - - - - - - - - - - - - G G R - - - - D S R S G S P H A R R
Chic  G H K G Y S K Q(G,H,G)T L S K I F K L G G K P(S,G,S,G,S)R S G S P V A R R
```

Amino acid sequences of myelin basic proteins derived from human (Hum), bovine (Bov), rabbit (Rab), guinea pig (GPig), rat (RatS), and chicken (Chic) central nervous system tissue. The rat BP used was the 14 kDa BP (rat small, or RatS) which has a deletion of residues 118–159, coded for by the 6th exon of the BP gene. Both the human 18.5 and 17.2 kDa forms of BP were used. The latter has a deletion of residues 107–117 (underlined), coded for by the 5th exon of the BP gene. The sequences are arranged such that homologous residues from each species are arranged vertically, so that they can easily be compared with one another. This system accommodates the deletions and additions that are found among the species, and allows for a total of 177 potential sites among the different molecules. The sequences of residues in parentheses have not been established.

The comparative amino acid sequences:

Human α1(II)	Bovine α1(II)	Bovine α1(I)	
gly pro met	[gly VAL met]	gly pro met	
gly pro met	gly pro met	[gly pro SER]	105
gly pro arg	gly pro arg	gly pro arg	
gly pro pro	gly pro pro•	[gly LEU pro•]	
gly pro ala	gly pro ala	[gly pro PRO•]	
gly ala pro	gly ala pro•	gly ala pro•	
gly pro gln	gly pro gln	gly pro gln	
gly phe gln	gly phe gln	gly phe gln	
gly asn pro	gly asn pro	[gly PRO pro]	
gly glu pro	gly glu pro•	gly glu pro•	
gly glu pro	gly glu pro•	gly glu pro•	132
gly val ser	gly val ser	[gly ALA ser]	
gly pro met	gly pro met	gly pro met	
gly pro arg	gly pro arg	gly pro arg	
gly pro pro	gly pro pro•	gly pro pro•	
gly pro pro	gly pro pro•	gly pro pro•	
gly lys pro	gly lys pro•	[gly lys ASN]	
gly asp asp	gly asp asp	gly asp asp	
gly glu ala	gly glu ala	gly glu ala	
gly lys pro	gly lys pro•	gly lys pro•	
gly lys ala	[gly lys SER]	[gly ARG PRO•]	162
gly glu arg	gly glu arg	gly glu arg	
gly pro pro	gly pro pro•	gly pro pro•	
gly pro gln	gly pro gln	gly pro gln	
gly ala arg	gly ala arg	gly ala arg	
gly phe pro	gly phe pro•	[gly LEU pro•]	
gly thr pro	gly thr pro•	[gly thr ALA]	
gly leu pro	gly leu pro•	gly leu pro•	
gly val lys	gly val lys•-glc-gla	[gly MET lys•-glc-gla]	
gly his arg	gly his arg	gly his arg	
gly tyr pro	gly tyr pro•	[gly PHE SER]	192
gly leu asp	gly leu asp	gly leu asp	
gly ala lys	gly ala lys•-glc-gla	gly ala lys•-glc-gla	
gly glu ala	gly glu ala	[gly ASP ala]	
gly ala pro	gly ala pro•	[gly PRO ALA]	
gly val lys	gly val lys	[gly PRO lys]	
gly glu ser	gly glu ser	[gly glu PRO•]	
gly ser pro	gly ser pro•	gly ser pro•	
gly glu asn	gly glu asn	gly glu asn	
gly ser pro	gly ser pro•	[gly ALA pro•]	
gly pro met	gly pro met	[gly GLN met]	222
gly pro arg	gly pro arg	gly pro arg	
gly leu pro	gly leu pro•	gly leu pro•	
gly glu arg	gly glu arg	gly glu arg	
gly arg thr	gly arg thr	[gly arg PRO•]	

Page 1

A 4

Column 1	Column 2	Column 3	
gly pro ala	gly pro ala	gly pro PRO*	
gly ala ala	gly ala ala	gly SER ala	
gly ala arg	gly ala arg	gly ala arg	
gly asn asp	gly asn asp	gly ASP asp	
gly gln pro	gly gln pro*	gly ALA VAL	
gly pro ala	gly pro ala	gly ALA ala	252
gly pro pro	gly pro pro*	gly pro pro*	
gly pro val	gly pro val	gly pro THR	
gly pro ala	gly pro ala	gly pro ala	
gly gly pro	gly gly pro*	gly PRO pro*	
gly phe pro	gly phe pro*	gly phe pro*	
gly ala pro	gly ala pro*	gly ala VAL	
gly ala lys	gly ala lys*-glc-gla	gly ala lys*-glc-gla	
gly glu ala	gly glu ala	gly glu GLY	
gly pro thr	gly pro thr	gly pro thr	282
gly ala arg	gly ala arg	gly PRO arg	
gly pro glu	gly pro glu	gly SER glu	
gly ala gln	gly ala gln	gly PRO gln	
gly pro arg	gly pro arg	gly VAL arg	
gly glu pro	gly glu pro*	gly glu pro*	
gly thr pro	gly thr pro*	gly PRO pro*	
gly ser pro	gly ALA pro*	gly PRO ALA	
gly pro ala	gly pro ala	gly ALA ala	
gly ala ser	gly ala ALA	gly PRO ALA	
gly asn pro	gly asn pro*	gly asn pro*	312
gly thr asp	gly ALA asp	gly ALA asp	
gly ile pro	gly ile pro*	gly GLU pro*	
gly ala lys	gly ala lys*	gly ala lys*	
gly ser ala	gly ser ala	gly ALA ASN	
gly ala pro	gly ala pro*	gly ala pro*	
gly ile ala	gly ile ala	gly ile ala	
gly ala pro	gly ala pro*	gly ala pro*	
gly phe pro	gly phe pro*	gly phe pro*	
gly pro arg	gly ALA arg	gly ALA arg	
gly pro pro	gly pro pro*	gly pro SER	
asp pro gln	GLY PRO THR	GLY PRO GLN	342
gly ala thr	gly ala SER	gly ala PRO	
gly pro leu	gly pro leu	gly pro PRO*	
gly pro lys	gly pro lys*	gly pro lys*	
gly gln thr	gly gln thr	gly ASN SER	
gly lys pro	gly lys pro	gly lys pro	
gly ile ala	gly ile ala	gly ALA PRO*	
gly phe lys	gly phe lys*	gly ASN lys*	
gly glu gln	gly glu gln	gly ASP THR	
gly pro lys	gly pro lys*	gly ALA lys*	372
gly glu pro	gly glu pro*	gly glu pro*	
gly pro ala	gly pro ala	gly pro THR	
gly pro gln	gly VAL gln	gly ILE gln	
gly ala pro	gly ala pro*	gly PRO pro*	

Column 1	Column 2	Column 3	
gly pro ala	gly pro ala	gly pro ala	
gly glu glu	gly glu glu	gly glu glu	
gly lys arg	gly lys arg	gly lys arg	
gly ala arg	gly ala arg	gly ala arg	
gly glu pro	gly glu pro*	gly glu pro*	
gly gly val	gly gly ALA	gly PRO THR	402
gly pro ile	gly pro ALA	gly LEU PRO*	
gly pro pro	gly pro pro*	gly pro pro*	
gly glu arg	gly glu arg	gly glu arg	
gly ala pro	gly ala pro*	gly GLY pro*	
gly asn arg	gly SER arg	gly SER arg	
gly phe pro	gly phe pro*	gly phe pro*	
gly gln asp	gly gln asp	gly ALA asp	
gly leu ala	gly leu ala	gly VAL ala	
gly pro lys	gly pro lys*	gly pro lys*	
gly ala pro	gly PRO pro*	gly PRO ALA	432
gly glu arg	gly glu arg	gly glu arg	
gly pro ser	gly SER PRO*	gly ALA PRO*	
gly leu ala	gly ALA VAL	gly PRO ALA	
gly pro lys	gly pro lys*	gly pro lys*	
gly ala asn	gly SER PRO*	gly SER PRO*	
gly asp pro	gly GLU ALA	gly GLU ALA	
gly arg pro	gly arg pro*	gly arg pro*	
gly glu pro	gly glu ALA	gly glu ALA	
gly leu pro	gly leu pro*	gly leu pro*	
gly ala arg	gly ala LYS*	gly ala LYS*	462
gly leu thr	gly leu thr	gly leu thr	
gly arg pro	gly arg pro*	gly SER pro*	
gly asp ala	gly asp ala	gly SER PRO*	
gly pro gln	gly pro gln	gly pro ASP	
gly lys val	gly lys val	gly lys THR	
gly pro ser	gly pro ser	gly pro PRO*	
gly ala pro	gly ala pro*	gly PRO ALA	
gly glu asp	gly glu asp	gly GLN ASN	
gly arg pro	gly arg pro*	gly arg pro*	
gly pro pro	gly pro pro*	gly pro pro*	492
gly pro gln	gly pro gln	gly pro PRO*	
gly ala arg	gly ala arg	gly ala arg	
gly gln pro	gly gln pro*	gly gln ALA	
gly val met	gly val met	gly val met	
gly phe pro	gly phe pro*	gly phe pro*	
gly pro lys	gly pro lys*	gly pro LYS	
gly ala asn	gly ala asn	gly ala ALA	
gly glu pro	gly glu pro*	gly glu pro*	
gly lys ala	gly lys ala	gly lys ala	
gly glu lys	gly glu lys*	gly glu ARG	522
gly leu pro	gly leu pro*	gly VAL pro*	
gly ala pro	gly ala pro*	gly PRO pro*	
gly leu arg			

Page 3

A6

gly leu pro
gly lys asp
gly glu thr
gly ala glu
gly pro pro
gly pro ala
gly pro ala
gly glu arg   552
gly glu gln
gly ala pro
gly pro ser
gly phe gln
gly leu pro
gly pro pro
gly pro pro
gly glu ala
gly lys pro
gly asp gln   582
gly val pro
gly glu ala
gly ala pro
gly leu val
gly pro arg
gly glu arg
gly phe pro
gly glu arg
gly ser pro
gly ala gln   612
gly leu gln
gly pro arg
gly leu pro
gly thr pro

gly thr asp	gly thr asp	gly ASN asp
gly pro lys	gly pro lys*	gly ALA lys*
gly ala ser	gly ala ALA	gly ASP ALA
gly pro ala	gly pro ala	gly ALA PRO*
gly pro pro		

               642

gly ala gln
gly pro pro
gly leu gln
gly met pro
gly glu arg
gly ala ala

gly ile ala	gly ile ala	gly LEU PRO*
gly pro lys	gly pro lys*	gly pro LYS
gly asp arg	gly asp arg	gly asp arg
gly asp val	gly asp val	gly asp ALA
gly glu lys	gly glu lys	gly PRO lys
gly pro glu	gly pro glu	gly ALA ASP

               672

gly pro pro
gly ser pro
gly pro ala
gly pro thr
gly lys gln
gly asp arg
gly glu ala
gly ala gln
gly pro met
gly pro ser
gly pro ala   1002
gly ala arg
gly ile gln
gly pro gln
gly pro arg
gly asp lys
gly glu ala
gly glu pro
gly glu arg
gly leu lys
gly his arg   1032
gly phe thr
gly leu gln
gly leu pro
gly pro pro
gly pro ser
gly asp gln
gly ala ser
gly pro ala
gly pro ser
gly pro arg   1062
gly pro pro
gly pro val
gly pro ser
gly lys asp
gly ala asn
gly ile pro
gly pro ile
gly pro pro
gly pro arg
gly arg ser   1092
gly glu thr
gly pro ala
gly pro pro
gly asn pro
gly pro pro
gly pro pro
gly pro pro
gly pro gly
_____
                                                                   1119

gly ala pro   gly ala pro   gly ala pro
gly lys asp
gly ala arg
gly leu thr
gly pro ile
gly pro pro
gly pro ala
gly ala asn
gly glu lys
gly glu val
gly pro pro
gly pro ala
gly ser ala
gly ala arg
gly ala pro
gly glu arg
gly glu thr
gly pro pro
gly pro ala
gly phe ala
gly pro pro
gly ala asp
gly gln pro
gly ala lys
gly glu gln
gly glu ala
gly gln lys
gly asp ala
gly ala pro
gly pro gln
gly pro ser
gly ala pro
gly pro gln
gly pro thr
gly val thr
gly pro lys
gly ala arg
gly ala gln
gly pro pro
gly ala thr
gly phe pro
gly ala ala
gly arg val
gly pro pro
gly ser asn
gly asn pro
gly pro pro
gly pro pro
gly pro ser

Column 2 (middle):

| gly ASP VAL |
| gly glu lys* |
| gly glu val |
| gly pro pro* |

gly gln pro
gly ala lys*

| gly GLY gln |
| gly glu ala |
| gly gln lys* |
| gly asp ala |
| gly ala pro* |

Column 3 (right):

| gly ALA PRO* |
| gly ASP LYS |
| gly glu ALA |
| gly pro SER |

     702

     732

| gly gln PRO* |
| gly ala LYS |
| gly GLU PRO* |
| gly ASP ala |
| gly ALA lys* |

gly asp ala
gly ala pro*

     762

     792

     822

gly lys asp
gly pro lys
gly ala arg
gly asp ser
gly pro pro
gly arg ala
gly glu pro
gly leu gln
gly pro ala
gly pro pro   852
gly glu lys
gly glu pro
gly asp asp
gly pro ser
gly ala glu
gly pro pro
gly pro gln
gly leu ala
gly gln arg
gly ile val   882
gly leu pro
gly gln arg
gly glu arg
gly phe pro
gly leu pro
gly pro ser
gly glu pro
gly gln gln
gly ala pro
gly ala ser   912
gly asp arg
gly pro pro
gly pro val
gly pro pro
gly leu thr
gly pro ala
gly glu pro
gly arg glu
gly ser pro
gly ala asp   942
gly pro pro
gly arg asp
gly ala ala
gly val lys
gly asp arg
gly glu thr
gly ala val
gly ala pro
gly ala pro

                                                                 972